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Synthesis and Properties of New Substituted 1,2,4-Triazoles: Potential Antitumor Agents

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Abstract—Cycloaddition of the reactive intermediates **4** with 1-(cyanomethyl)benzotriazole (**5**) and its N-2 isomer **9** furnished, after spontaneous rearrangements, the 1,2,4-triazole derivatives **8** and **10**. Analogously, reaction of **4** with ethyl cyanoacetate lead to the 1,3,5-trisubstituted 1,2,4-triazoles **12**, which gave on treatment with hydrazine the corresponding hydrazides **13**. Treatment of **13d** with galactose or phenyl isothiocayanate gave the 1-D-galactose-acylhydrazone 14 and the 1,2,4-triazole derivative **15**, respectively. Compounds **8c**; **10b,c**; **13a,c** and **14** were selected for the antitumor screening, whereby **8c**, **13a**, and **13c** showed remarkable activity against leukemia, ovarian, renal and lung cancers (**8c** with GI_{50} of 0.70 μM, 0.07 μM against leukemia (CCRF-CEM and RPMI-8226), 0.02 μM against ovarian (OVCAR-3) and 0.60 μM against renal (CARKI-1) and lung cancers, respectively).

Introduction

The 1*H*-benzotriazol compounds possess important pharmacological activities such as anti-inflammatory,[1] antiviral,[2] antifungal,[3] antineoplastic[4] and antidepressant[5] effects. Such compounds are Metronidazole and analogues used as drugs for the treatment of trichomoniasis and amoebicis, as well as Fluconazole and Miconazole, which are useful antifungal agents.[6–9] Recently, Moon et al.[10] reported the synthesis and biological activity of the new benzotriazolium Cephalosporins **1**, which exhibited remarkable activity against *Pseudomonas aeruginosa* and other bacteria, depending on the group attached to the benzotriazole backbone. Furthermore, some 2-alkyl-5-aminobenzotriazoles **2**[11,12] showed interesting antimicrobial activities against Gram-positive, Gram-negative bacteria and *Candida albicans*. More examples, such as 1-and 2-[3-(1-piperazinyl)propyl]-benzotriazoles showed in vitro remarkable antiserotonergic, antiadrenergic and antihistaminic activity, as well as in vivo analgesic action.[13] In addition, the benzotriazoles **5** and **9** are themself active against Leishmania.[14] In respect with the potential activity of these molecules and the structurally related benzo-fused-imidazoles (rifaximin,[15] as antineoplastic and anticancer agents), we focused our interest in designing a novel type of trisubstituted 1,2,4-triazoles bearing methyl benzotriazole residues in the 2-positions, starting with **5** and its N-2 isomer **9,** to give compounds **8** and **10**, respectively. These compounds can be considered as alkylating agents interfering with the metabolism, in vivo, as demonstrated in malignant diseases such as haematological and solid malignancies,[16] for which Dacarbazine®[17] is a good example. In the same respect, some substituted hydrazine compounds, like Procarbazine®,[19] was found to possess antineolpastic activity particularly in the treatment of Hodgkins' disease.[18] These biological data prompted us to synthesize new trisubstituted-1,2,4-triazoles bearing an acetohydrazide functionality which was further modified by a sugar or a 2-(1,2,4-triazolylthione) derivative. Recently, Katritzky et al.[12–27] and other laboratories[28–32] reported the synthesis of numerous examples of benzotriazole bearing diverse heterocycles.

Results and Discussion

Recently, the short-lived reactive intermediates 1-(chloroalkyl)-1-aza-2-azoniaallene salts **4** were used by Jochims and co-workers[32] in the synthesis of various 1,2,4-triazole compounds via cycloaddition reaction with various unsaturated precursors in the presence of

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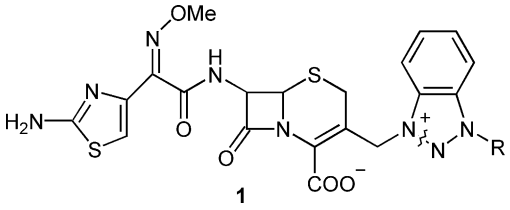

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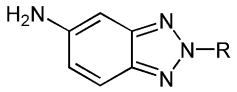
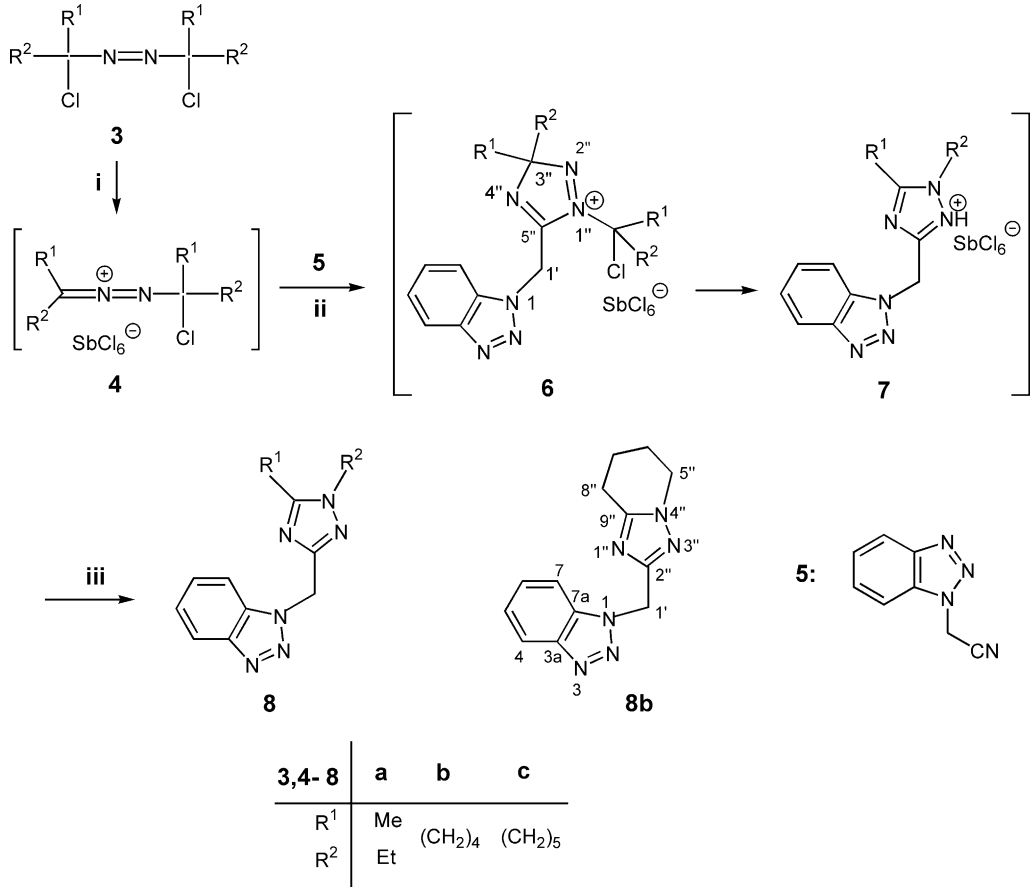


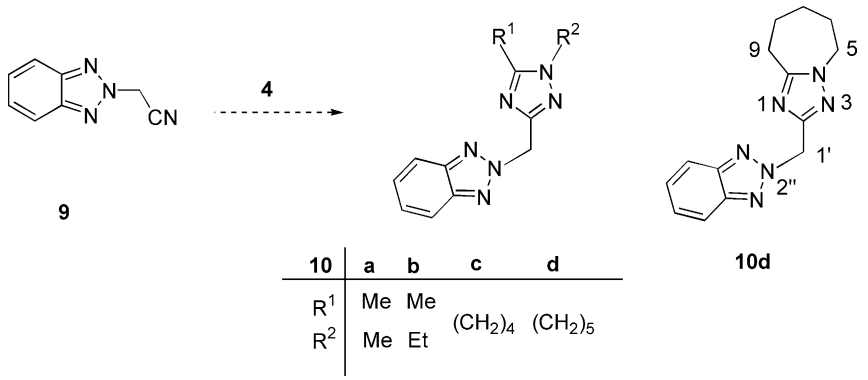
2: R = Me, cyclopentyl, cyclohexyl

$SbCl_5$. In our recent work, these cations have been utilized in the synthesis of new type of 1,2,4-triazole compounds such as C-ribonucleosides,[33,34] acyclic C-nucleosides[35] and homo-C-analogues,[35] C-nucleosides of D-mannose,[36] as well as attached to thymine,[37] phthalimide,[38] indole, and quinolone.[39] In the present work, 1-(cyanomethyl)benzotriazole (**5**) and its N-2 ismer **9**, were selected for the cycloaddition reaction. These nitriles were prepared by different methods already, but the choosen synthetic route via the the benzotriazole with chloroacetonitrile in the presence of Et_3N gave, after chromatographic separation, **5** in 20% and **9** 72% yield,[28] whereas Katritzky et al.[22] obtained **5** exclusively in 72% from condensation of sodium 1*H*-benzotriazolide with chloroacetonitrile in the presence of 8-crown-6. The dichlorides **3** were obtained from their bis-hydrazone analogues by chlorination and followed by treatment with $SbCl_5$ at approximately −60 °C to yield the reactive intermediate salts **4**. Addition of the nitriles in abs. CH_2Cl_2 at −30 °C resulted in a color change of the solution from orange to brown, indicating that cycloaddition reaction had occurred with the formation of unseparable intermediate 5-(benzotriazol-1-ylmethyl)-3*H*-1,2,4-triazolium hexachloroantimonates **6**. Interestingly an increase in the temperature to 23 °C caused [1,2] migration[40,41] of the alkyl group (R^2) from C-3 to N-2 and elimination of the ($CClR^1R^2$) group leading to the 1,2,4-triazolium salts **7**. The target compounds **8** were obtained[41] in 70, 78, 75% yield, respectively, by direrct neutralisation of the triazolium salts **8a-c**, in situ, with aqueous $NaHCO_3$[40,42] and aqueous NH_3 solutions (Scheme 1). Similarly, **10a–d** were obtained successively from the cycloaddition of **4** with the nitrile **9** in 75–80% yields (Scheme 2).

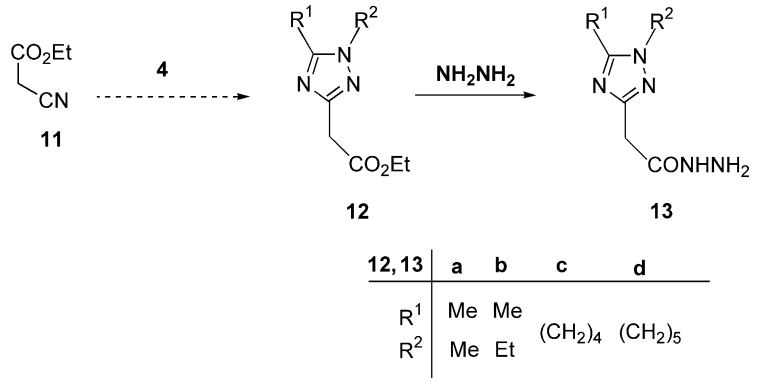
Furthermore, the nitrile **11** added to the reactive cumulene **4** under the same conditions described above, affording ethyl 1,2,4-triazol-3-ylacetate analogues **12a–d** in 78, 70, 80 and 86% yield, respectively. Treatment of **12** with hydrated hydrazine at 23 °C for 72 h lead to the corresponding hydrazide analogues **13a–d** in 80, 85, 85 and 90% yield, respectively (Scheme 3).



Scheme 1. Reagents and conditions: (i) $SbCl_5$, CH_2Cl_2, −60 °C; (ii) CH_2Cl_2, −60 to 23 °C; (iii) $NaHCO_3$, NH_3, MeCN, 0 °C, 2 h.



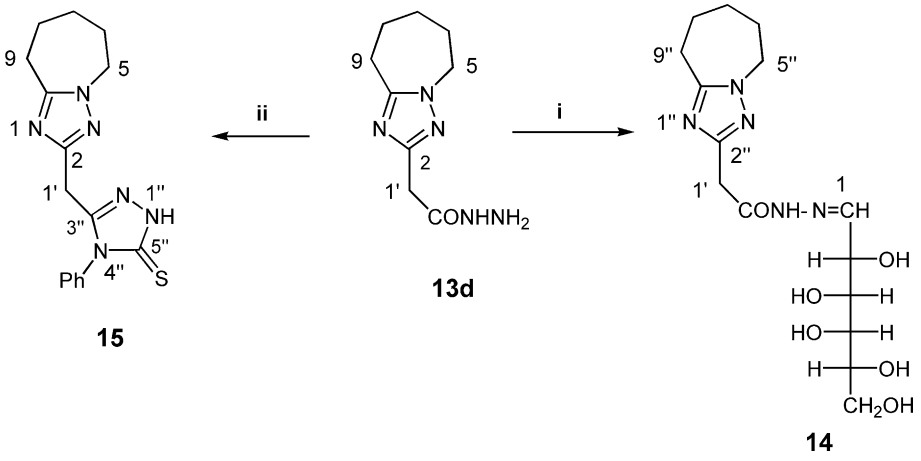
Scheme 2.



Scheme 3.

Compound **13d** was further modified by the reaction with galactose under reflux to give the acetohydrazide derivative **14** in 80% yield. Alternatively, the reaction of **13d** with phenyl isothiocyanate by heating under reflux temperature for 4 h gave the crude semithiocarbazide, was subsequently heated with 5% NaOH solution with reflux to give **15** (50%) (Scheme 4).

The structures of the newly prepared compounds were determined by their ^{1}H, ^{13}C NMR and by mass spectra. Compounds **8b** and **10c** were selected for further study via their DFQ-COSY[43] as well as HMQC and HMBC[44] spectroscopic measurements. The HMQC spectrum of **8b** showed a $^{2}J_{C,H}$ heteronuclear correlation of C-2″ (δ_C 156.3) to CH_2-1′ protons (δ_H 5.54). The spectrum was characterized also by two correlations: $^{2}J_{C,H}$ of C-9″ (δ_C 156.3) to CH_2-6″ (δ_H 1.50), as well as $^{3}J_{C,H}$ correlation of C-9″ to CH_2-5″ (δ_H 4.08). The formation of **8c** was proven further by the ROE between CH_2-1′ and H-7a of the aromatic ring. Furthermore, the signals at δ_C 47.0, 45.8 and 20.2 were assigned to C-1′, C-5″ and C-8″ as they have a cross peaks to δ_H 5.54, 4.08 and 2.78 of CH_2-1′, CH_2-5″ and CH_2-8″, respectively, in the HMQC spectrum. Compound **10d** was selected for the spectroscopic study, since CH_2-1′ at δ_H 5.90 showed heteronuclear correlation to C-10 (δ_C 155.2), with disappearance of such correlation between CH_2-1′ and the aromatic protons, indicating for the N-2 substitution.



Scheme 4. Reagents and conditions: (i) D-galatose, reflux; (ii) PhSCN, 5% NaOH, reflux.

The structures of **12** and **13** were established by homo- and heteronuclear NMR spectroscopic methods and by mass spectra. C-3′s and C-5′s at **12** and **13** were assigned, and CH_2-1′ protons appeared as singlets in the region δ_H 3.65–3.76. The CH_2-1′ protons of **12a,b** and **13a,b** were found at δ_H 2.39, 2.35, 2.43 and 2.35, respectively, showed a $^2J_{C,H}$ correlations in their HMQC spectra to C-3′s, and $^3J_{C,H}$ correlation to C-5′s. CH_2-5–CH_2-8 protons of the pyridine triazole ring of **12c** and **13c** were appeared mostly as a tripltes by their HMQC NMR spectra. The ^{13}C NMR spectra of **12c** contained the resonance signals C-2 and C-9 of the triazole ring at δ_C 156.2 and 157.7 respectively, whereas the signals resonated at δ_C 155.0 and 152.5 were attributed to the same atoms of **13c**, respectively. Compound **12d** showed the same signals at δ_C 157.7 and 154.9, attributed to C-2, and C-10, respectively, since the signals at δ_C 157.9 and 155.3 were assigned to the same atoms in **13d**, respectively. Compound **12b** was chosen for the HMQC analysis. Thus, C-3 at δ_C 156.0 is identified from its correlation to the ethyl group and to one methylene group (CH_2-1′) at δ_H.3.68. The structural assignments of **14** follows from the mass spectra and the 2D NMR spectrum. The HMQC spectrum showed a $^2J_{C,H}$ correlation between CH-1′ at δ_H 3.40 and C=O at δ_C 172.5 as well as C-2″ at δ_C 157.1. The ten azepine protons of the triazole ring were identified mostly as a *pseudo* triplets (pt). H-1 (C*H*=N) appeared as a doublet at δ_H 3.78 ($J_{1,2} < 1.0$ Hz), due to the galactose configuration. The other sugar protons were analysed by their DFQ-COSY and HMQC NMR spectra after exchanging with D_2O. Similarly **15** was identified by the homo- and heteronuclear spectra. CH_2-1′ (δ_H 3.83) showed two $^2J_{C,H}$ correlations to: C-3″ (δ_C 149.4) and C-2 (δ_C 157.3). The resonance at δ_C 167.9 was attributed to the (C=S) group.

Table 1. In vitro model primary anticancer data[a,b] for some of the new compounds at concentration (10^{-4} M)

Compd.	Growth percentages (GP)									Activity
	I	II	III	IV	V	VI	VII	VIII	IX	
8c	−5[c]	34	33	19	14	−15[c]	4	41	22	Active
10b	58	64	69	73	31	86	63	63	67	Inactive
10c	39	71	82	75	76	69	85	82	75	Inactive
13a	36	88	72	82	81	79	70	84	70	Inactive
13c	58	36	71	69	59	71	76	73	83	Inactive
15	—	137	—	124	—	—	—	—	103	Inactive

[a]I. leukemia (RPMI-8226); II. non-small cell lung cancer (NCI-H522); III. colon cancer (HCC-2998); IV. CNS cancer (SF-539); V. melanoma (UACC-62); VI. ovarian cancer (OVCAR-1); VII. Renal cancer (CAKI-1); VIII. prostate cancer (DU-145); IX. breast cancer (MCF7).
[b]Results for each test agent are reported as the pecent age growth of the treated cell compared to the untreated control cells.
[c]Negative number indicates cell kill at a concentration 10^{-4} M.

Antitumor activity

Compounds **8c**, **10b,c**, **13a,c** and **15** were selected by the the Developmental Therapeutic Program Division of Cancer Treatment, United States, National Cancer Institute (NCI) for the antitumor screening in vitro against a panel consisting of 60 human tumor cell lines, derived from nine cancer types including: leukemia, non-small cell lung, colon, CNS, melanoma, ovarian, renal, prostate, and breast cancers. Compounds were tested at five fold dilutions from a maximum concentration of 10^{-4} M. The activity of each compound is expressed as the GI_{50}, TGI, and LC_{50} values which represent the molar drug concentrations required to cause half growth inhibition, total growth inhibition or a net 50% loss of initial cells at the end of the incubation period, respectively.[45–47] Subpanel and full panel mean-graph midpoint values (MG-MID) are the average of individual and default values of all cell lines in the subpanel or the full panel, respectively.[45–47] The results are displayed in Table 1. Compound **8c** is considred active against leukemia (RPMI-8226) and ovarian (OVCAR-1), where it showed remarkable low pecentage growth of $\log_{10}$ concentration = −5.0 and −15; respectively, since the negative value indicates cells killed at concentration 10^{-4} M. In addition, the same compound showed remarkable activity against individual cell lines (data not shown), e.g., with GI_{50} of 0.70 μM, 0.07 μM against leukemia (CCRF-CEM and RPMI-8226), 0.02 μM against ovarian (OVCAR-3) and 0.60 μM against renal cancer (CARKI-1), respectively, while compounds **10b**, **10c**, **13a**, **13c** and **15** did not showed such activity.

The ratio obtained by the compound's full panel MG-MID (μM) by its individual subpanel MG-MID (μM) is considered as an indicator of the compound's selectivity (Δ), which means ratios: <3.0, with low selectivity, >3.0 and <6.0, refer to a moderate selectivity, while ratios >6 indicate high selectivity against the corresponding subpanel.[46,48] All the compounds in the pre-

Table 2. Median growth inhibitory concentration (GI_{50}, μM) in vitro subpanel tumor cell lines[a,b,c]

Compd	Subpanel tumor cell lines selectivity analysis (differential cellular sensitivity) (Δ)[d]									MG-MID[e]
	I	II	III	IV	V	VI	VII	VIII	IX	
8c	23.2 (0.75)	62.5 (2.01)	68.5 (2.21)	30.9 (1.0)	55.9 (0.51)	34.8 (1.12)	46.0 (1.48)	54.7 (1.76)	66.6 (2.15)	31.0
Melphalan	20.1	38.5	42.1	17.1	31.9	43.0	34.4	34.7	39.2	27.1

[a]I. leukemia; II. non-small cell lung cancer; III. colon cancer; IV. CNS cancer; V. melanoma; VI. Ovarian cancer; VII. Renal cancer; VIII. prostate cancer; IX. breast cancer.
[b]GI_{50}: concentration giving 50% inhibition.
[c]Compounds **10b**, **10c**, **13a**, **13c**, **15** with $GI_{50} > 100$ μM.
[d]MG-MID full panel mean-graph mid-point.
[e]The reported data represent the ratio obtained by the compound's full panel MG-MID (μM) by its individual subpanel MG-MID (μM) line, which indicate the selectivity analysis (Δ) is considered low if <3, moderate if >3 and high if >6.

sent study proved to be non-selective towared all the cell tumor lines (Table 2), since showed (Δ) values <3.0. An example for such non-selectivity demonstrated by the following compounds: **8c**, which exhibited broad antitumor activity against the nine tumor subpanels, gave ratios of (Δ) 0.75–2.21; **13a** and **13c** with (Δ) 1.75, and 2.50 against leukemia, respectively.

When comparing the antitumor activity of **8c** with the known antineoplastic agent Melphalan,[49] it has been found that the GI_{50} (MD-MID) of Melphalan is 27.1 versus 31.0 of **8c,** indicating both compounds have nearly the same activity, but Melphalan is a more selective antitumor agent.

In conclusion, this study indicates that N-1-benzotriazole carrying a triazolo-pyridine substituent (**8c**) exhibited good activity against certain tumor cell lines, whereas the N-2 isomers (**13a**, **13c**) did not showed any effect. The hydrazide derivatives **13b**, **13c** as well as the structurally more complex compound **15** showed no effect on the nine tumor cell lines.

Experimental

General procedure[33–38]

Preparation of 1,3-5-trisubstituted 1,2,4-triazoles bearing a 3-(benzotriazolylmethylene) or 3-(ethoxycarbonylmethyl) group (8, 10 and 12). A solution of $SbCl_5$ (1.5 g, 4.0 mmol) in CH_2Cl_2 (10 mL) was added dropwise to a stirred, cold solution (−60 °C) of **3**[32] (4.0 mmol) in dry CH_2Cl_2 (30 mL) followed directly by the addition of the nitriles **5**, **9** or **11** (3.0 mmol). After stirring for 2 h at −60 °C, 1 h at 0 °C, and 10 min at 23 °C, pentane (60 mL) was added and the residue was filtered and dissolved in CH_3CN (60 mL). The solution was treated after cooling to 0 °C with an aqueous solutions of $NaHCO_3$ (3.36 g, 40 mmol) in H_2O (40 mL) and NH_3 solution (4.0 mmol) were added and the mixture was stirred at 23 °C for 2 h. The mixture was evaporated and the aqueous phase was extracted with CH_2Cl_2 (3×50 mL). The combined organic exctracts were dried (Na_2SO_4), filtered and the filtrate was evaporated to dryness. The residue was recrystallized from CH_2Cl_2 to give the pure desired products **8**, **10** and **12**.

1-[1-Ethyl-5-methyl-1*H*-1,2,4-triazole-3-yl(methylene)]-1*H*-benzotriazole (8a). From **3a** (1.06 g) and the nitrile **5**. Yield: 0.85 g, 70%; oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.20–7.27 (m, 4H, Ar–H); 5.60 (s, 2H, CH_2-1′); 4.10 (q, 2H, J 7.0 Hz, $N^{5''}$–*CH_2*CH_3); 2.35 (s, 3H, C-5-Me); 1.36 (t, 3H, $N^{5''}$–CH_2*CH_3*). ^{13}C NMR ($CDCl_3$) δ 156.3 (C-3″); 152.5 (C-5″); 146.0, 127.3, 123.9, 119.7, 112.5, 110.1 (Ar); 45.7 (C-1′); 43.4 ($N^{5''}$–*CH_2*CH_3); 14.8 ($N^{5''}$–CH_2*CH_3*); 11.7 (C-5″-*Me*).). Anal. calcd for $C_{12}H_{14}N_6$ (242.3): C, 59.49; H, 5.82; N, 34.69. Found: C, 59.35; H, 5.79; N; 34.57; *m*/*z* (FAB) 243 $(MH)^+$.

1-[5,6,7,8-Tetrahydro-[1,2,4]triazolo[1,5-a]pyridine-2-yl (methylene)]-1*H*-benzotriazole (8b). From **3b** (1.17 g) and the nitrile **5**. Yield: 0.95 g, 78%; oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.11–7.22 (m, 4H, Ar–H); 5.54 (s, 2H, CH_2-1′); 4.08 (t, 2H, *J* = 6.0 Hz, CH_2-5″); 2.78 (t, 2H, *J* = 6.0 Hz, CH_2-8″); 2.10–1.50 (m, 4H, CH_2-6″, CH_2-7″). ^{13}C NMR ($CDCl_3$) δ 156.3 (C-2″); 152.5 (C-9″); 146.2, 127.3, 123.9, 119.9, 112.4, 108.7 (Ar); 47.0 (C-1′); 45.8 (C-5″); 24.0 (C-6″); 23.5 (C-7″); 20.2 (C-8″). Anal. calcd for $C_{13}H_{14}N_6$ (254.3): C, 61.40; H, 5.55; N, 33.05. Found: C, 61.20; H, 5.49; N; 32.91; *m*/*z* (EI) 254 $(M)^+$.

2-[*H*-Benzotriazole-1-yl(methylene)]-6,7,8,9-tetrahydro-5*H*-[1,2,4]triazolo[1,5-a]azepine (8c). From **3c** (1.19 g) and the nitrile **5**. Yield: 1.0 g, 75%; brown oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.15–7.28 (m, 4H, Ar–H); 5.59 (s, 2H, CH_2-1′); 4.13 (t, 1H, *J* = 5.0 Hz, CH_2-5); 2.79 (t, 2H, *J* = 2.5 Hz, CH_2-9); 2.28 (t, 2H, *J* = 5.0 Hz, CH_2-7); 1.78 (t, 2H, *J* = 4.5 Hz, CH_2-6); 1.65 (t, 2H, *J* = 5.5 Hz, CH_2-8). ^{13}C NMR ($CDCl_3$): δ 158.4 (C-2); 155.5 (C-10); 146.1 (C-3a″), 127.3 (C-7a″), 123.8, 119.8, 112.4, 110.1 (Ar); 51.2 (C-1′); 45.6 (C-5); 30.1 (C-7); 27.3 (C-6, C-9); 24.7 (C-8). Anal. calcd for $C_{14}H_{16}N_6$ (268.3): C, 62.67: H, 6.01; N, 31.32. Found: C, 62.53; H, 5.98; N; 31.22; *m*/*z* (FAB) 269 $(MH)^+$.

2-[1,5-Dimethyl-1*H*-1,2,4-triazole-3-yl(methylene)]-2*H*-benzotriazole (10a). From **3a** (1.06 g) and the nitrile **9**. Yield: 0.90 g, 75%; brown oil. ^{1}H NMR ($CDCl_3$) δ 7.88–7.32 (m, 4H, Ar–H); 5.90 (s, 2H, CH_2-1′); 3.76 (s, 3H, NMe); 2.38 (s, 3H, C-5-Me). ^{13}C NMR ($CDCl_3$) δ 156.3 (C-3″); 152.5 (C-5″); 146.0, 127.3, 123.9, 119.7, 112.5, 110.1 (Ar); 45.7 (C-1′); 43.4 ($N^{5''}$–*CH_2*CH_3); 14.8 ($N^{5''}$–CH_2*CH_3*); 11.7 (C-5-*Me*). Anal. calcd for $C_{12}H_{14}N_6$ (242.3): C, 59.49; H, 5.82; N, 34.69. Found: C, 59.35; H, 5.79; N; 34.27; *m*/*z* (FAB) 243 $(MNa)^+$.

2-[1-Ethyl-5-methyl-1*H*-1,2,4-triazole-3-yl(methylene)]-2*H*-benzotriazole (10b). From **3b** (1.06 g) and the nitrile **9**. Yield: 0.91 g, 75%; brown semi-solid. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.88–7.25 (m, 4H, Ar-H); 5.88 (s, 2H, CH_2-1′); 4.00 (q, 2H, J 7.0 Hz, $N^{5''}$–*CH_2*CH_3); 2.37 (s, 3H, C-5-Me); 1.38 (t, 3H, $N^{5''}$–CH_2*CH_3*). ^{13}C NMR ($CDCl_3$) δ 156.0 (C-3″); 152.3 (C-5″); 144.3 (2x), 126.0 (2x), 123.9, 117.9 (2x) (Ar); 53.4 (C-1′); 43.1 ($N^{5''}$–*CH_2*CH_3); 14.6 ($N^{5''}$–CH_2*CH_3*); 11.5 (C-5-*Me*). Anal. calcd for $C_{12}H_{14}N_6$ (242.3): C, 59.49; H, 5.82; N, 34.69. Found: C, 59.24; H, 5.75; N; 34.52.; *m*/*z* (EI) 242 $(M)^+$.

2-[5,6,7,8-Tetrahydro-[1,2,4]triazolo[1,5-a]pyridine-2-yl (methylene)]-2*H*-benzotriazole (10c). From **3c** (1.17 g) and the nitrile **9**. Yield: 1.02 g, 80%; brown semi-solid. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.951–7.25 (m, 4H, Ar–H); 5.93 (s, 2H, CH_2-1′); 4.07 (t, 2H, *J* = 6.0 Hz, CH_2-9″); 2.93 (t, 2H, *J* = 6.0 Hz, C-6″); 2.32 (dt, 2H, *J* = 6.1 Hz, CH_2-7″); 2.19 (t, 2H, *J* = 6.1 Hz, CH_2-8″). ^{13}C NMR ($CDCl_3$) δ 156.9 (C-2″); 153.8 (C-9″); 144.6 (2x), 126.3 (2x), 118.2 (2x) (Ar); 57.2 (C-1′); 47.6 (C-5″); 23.6 (C-7″); 22.6 (C-6″); 20.7 (C-8″). Anal. calcd for $C_{13}H_{14}N_6$ (254.3): C, 61.40; H, 5.55; N, 33.05. Found: C, 61.31; H, 5.49; N; 32.88; *m*/*z* (EI) 254 $(M)^+$.

2-[2*H*-Benzotriazol-2-yl(methylene)]-6,7,8,9-tetrahydro-5*H*-[1,2,4]-triazolo[1,5-a]azepine (10d). From **3d** (1.19 g) and the nitrile **9**. Yield: 1.0 g, 75%; mp 128–131 °C. ^{1}H

NMR (600 MHz, $CDCl_3$) δ 7.89–7.20 (m, 4H, Ar–H); 5.90 (s, 2H, CH_2-1′); 4.20 (t, 1H, $J=5.0$ Hz, CH_2-5); 2.88 (t, 2H, $J=2.5$ Hz, CH_2-9); 1.82 (t, 2H, $J=4.9$ Hz, CH_2-7); 1.71 (t, 2H, $J=4.5$ Hz, CH_2-6); 1.62 (t, 2H, $J=5.5$ Hz, CH_2-8). ^{13}C NMR ($CDCl_3$) δ 158.5 (C-2); 155.2 (C-10); 144.6 (2x), 126.3 (2x), 118.2 (2x), (Ar); 53.5 (C-1′); 51.3 (C-5); 30.2 (C-7); 27.4 (C-6); 27.7 (C-9), 24.7 (C-8). Anal. calcd for $C_{14}H_{16}N_6$ (268.3): C, 62.67: H, 6.01; N, 31.32. Found: C, 62.49; H, 5.95; N, 31.12; *m/z* (EI) 268 $(M)^+$.

1,5-Dimethyl-1*H*-1,2,4-triazole-3-acetic acid ethyl ester (12a). From **3a** (0.73 g) and the nitrile **11**. Yield: 0.43 g, 78%; mp 91–93 °C. ^1H NMR ($CDCl_3$): δ 4.16 (q, 3H, $J=7.0$ Hz, CH_2CH_3); 3.75 (s, 2H, CH_2-1′); 3.68 (s, 3H, NMe); 2.39 (s, 3H, C-5-Me); 1.23 (t, 3H, CH_2CH_3). ^{13}C NMR ($CDCl_3$): δ 169.6 (C=O); 156.0 (C-3); 152.0 (C-5); 61.1 (CH_2CH_3); 34.9 (N^5–CH_3); 34.3 (C-1′); 14.0 (CH_2CH_3); 11.7 (C-5-*Me*). Anal. calcd for $C_8H_{13}N_3O_2$ (183.2): C, 52.45; H, 7.15; N, 22.94. Found: C, 52.22; H, 7.01; N, 21.81. MS: *m/z* (%) 183 (M^+) (50).

1-Ethyl-5-methyl-1*H*-1,2,4-triazole-3-acetic acid ethyl ester (12b). From **3b** (0.88 g) and the nitrile **11**. Yield: 0.41 g, 70%; mp 79–82 °C. ^1H NMR ($CDCl_3$): δ 4.15 (q, 2H, $J=7.0$ Hz, OCH_2CH_3); 4.02 (q, 2H, $J=7.0$ Hz, $N^5CH_2CH_3$); 3.68 (s, 2H, CH_2-1′); 2.35 (s, 3H, C_5-Me); 1.39 (t, 3H, N^5–CH_2CH_3); 1.22 (t, 3H, OCH_2CH_3). ^{13}C NMR ($CDCl_3$): δ 169.6 (C=O); 156.0 (C-3); 151.8 (C-5); 61.0 (OCH_2CH_3); 43.1 (N^5–CH_2CH_3); 34.4 (CH_2-1′); 14.8 (N^5–CH_2CH_3); 14.0 (OCH_2CH_3); 11.7 (C-5-*Me*). Anal. calcd for $C_9H_{15}N_3O_2$ (197.2): C, 54.81; H, 7.67; N, 21.30. Found: C, 54.60; H, 7.59; N, 21.12; *m/z* (EI) 197 $(M)^+$.

5,6,7,8-Tetrahydro-[1,2,4]triazolo[1,5-a]pyridine-2-acetic acid ethyl ester (12c). From **3c** (0.94 g) and the nitrile **11**. Yield: 0.50 g, 80%; mp 72–75 °C. ^1H NMR ($CDCl_3$): δ 4.20 (q, 2H, $J=7.0$ Hz, OCH_2CH_3); 4.14 (t, 2H, $J=6.0$ Hz, CH_2-5); 3.76 (s, 2H, CH_2-1′); 2.90 (t, 2H, $J=6.0$ Hz, CH_2-8); 2.19, 1.99 (m, 4H, CH_2-6, CH_2-7); 1.30 (t, 3H, OCH_2CH_3). ^{13}C NMR ($CDCl_3$): δ 169.6 (C=O); 156.5 (C-2); 153.0 (C-9); 61.0 (OCH_2CH_3); 46.6 (C-5); 34.4 (C-1′); 23.3 (C-7); 22.6 (C-6); 20.2 (C-8); 13.9 (OCH_2CH_3). Anal. calcd for $C_{10}H_{15}N_3O_2$ (209.2): 57..40; H, 7.23; N, 20.08. Found: C, 57.24; H, 7.11; N, 10.82; *m/z* (EI) 209 $(M)^+$.

6,7,8,9-Tetrahydro-5*H*-[1,2,4]triazolo[1,5-a]azepine-2-acetic acid ethyl ester (12d). From **3d** (1.05 g) and the nitrile **11**. Yield:0.57 g, 85%; mp 65–68 °C. ^1H NMR ($CDCl_3$): δ 4.18 (q, 2H, $J=7.0$ Hz, OCH_2CH_3); 4.10 (t, 2H, CH_2-5); 3.65 (s, 2H, CH_2-1′); 2.88 (t, 2H, $J=3.2$ Hz, CH_2-9); 1.84 (m, 2H, CH_2-7); 1.72 (m, 4H, CH_2-6, CH_2-8); 1.24 (t, 3H, OCH_2CH_3). ^{13}C NMR ($CDCl_3$): δ 169.6 (C=O); 157.7 (C-2); 154.9 (C-10); 61.0 (OCH_2CH_3); 50.9 (C-5); 34.3 (C-1′); 30.1 (C-7); 27.3 (C-6, C-9); 24.7 (C-8); 14.0 (OCH_2CH_3). Anal. calcd for $C_{11}H_{17}N_3O_2$ (223.3): C, 59.17; H, 7.67; N, 18.82. Found: C, 58.93; H, 7.57; N, 18.68; *m/z* (EI) 223 $(M)^+$.

Preparation of the acetic acid hydrazide derivatives of 1,5-disubstituted-1,2,4-triazoles (13). To a stirred suspension of **12** (5.46 mmol) in MeOH (30 mL) was added hydrated hydrazine (0.86 g, 25 mmol) at 23° C. After stirring with EtOH (50 mL) for 72 h, the suspension was evaporated to dryness and the residue was washed with Et_2O (50 mL), filtered and recrystallized from EtOH to give the desired products **9a–d**, respectively, as yellow crystals.

1,5-Dimethyl-1*H*-1,2,4-triazole-3-acetic acid hydrazide (13a). From **12a** (1.0 g). Yield: 0.74 g, 80%; mp 110–111 °C. ^1H NMR ($CDCl_3$): δ 8.37 (bs, 1H, NH); 3.78 (s, 2H, CH_2-1′); 3.67 (s, 3H, NMe); 2.43 (s, 3H, C-5–Me). ^{13}C NMR ($CDCl_3$): δ 168.7 (C=O); 156.7 (C-3); 152.8 (C-5); 35.0 (N^5-CH_3); 34.4 (C-1′); 11.7 (C-5–*Me*). Anal. calcd for $C_6H_{11}N_5O$ (169.2): C, 42.60; H, 6.55; N, 41.39. Found: C, 42.41; H, 6.50; N, 41.15; *m/z* (FAB) 192 $(MNa)^+$.

1-Ethyl-5-methyl-1*H*-1,2,4-triazole-3-acetic acid hydrazide (13b). From **12b** (1.0 g). Yield: 0.79 g, 85%; mp 101–104° C. ^1H NMR ($CDCl_3$): δ 8.34 (s, 1H, NH); 6.35 (bs, 2H, NH_2); 4.05 (q, 2H, $J=6.0$ Hz, OCH_2CH_3); 4.02 (q, 2H, $J=7.0$ Hz, N^5–CH_2CH_3); 3.68 (s, 2H, CH_2-1′); 2.35 (s, 3H, C-5–Me); 1.39 (t, 3H, N^5–CH_2CH_3). ^{13}C NMR ($CDCl_3$): δ 169.6 (C=O); 156.0 (C-3); 151.8 (C-5); 43.1 (N^5-CH_2CH_3); 34.4 (CH_2-1′); 14.8 (N^5–CH_2CH_3); 11.7 (C_5–*Me*). Anal. calcd for $C_7H_{15}N_3O$ (183): C, 54.81; H, 7.67; N, 21.30. Found: C, 54.60; H, 7.59; N, 21.12; *m/z* (EI) 183 $(M)^+$.

5,6,7,8-Tetrahydro-[1,2,4]-triazolo[1,5-*a*]pyridine-2-acetic acid hydrazide (13c). From **12c** (1.0 g). Yield: 0.79 g, 85%; mp 101–104 °C. ^1H NMR ($CDCl_3$): δ 8.34 (bs; 1H, NH); 6.35 (bs, 2H, NH_2); 4.05 (t, 2H, $J=6.0$ Hz, CH_2-5); 3.64 (s, 2H, CH_2-1′); 2.82 (t, 2H, $J=6.0$ Hz, CH_2-8); 2.05, 1.97 (m, 4H, CH_2-6, CH_2-8). ^{13}C NMR ($CDCl_3$): δ 166.0 (C=O); 155.0 (C-2); 152.5 (C-9); 46.6 (C-5); 34.5 (C-1′); 24.1 (C-7); 22.0 (C-6); 20.0 (C-8). Anal. calcd for $C_8H_{13}N_5O$ (195.2): C, 49.22; H, 6.71; N, 35.87. Found: C, 49.01; H, 6.64; N, 35.64; *m/z* (FAB) 218 $(MNa)^+$.

6,7,8,9-Tetrahydro-5*H*-[1,2,4]-triazolo[1,5-*a*]azepine-2-acetic acid hydrazide (13d). From **12d** (1.0 g). Yield: 0.84 g, 90%; mp 114–117 °C. ^1H NMR ($CDCl_3$): δ 8.34 (bs, 1H, NH); 6.35 (bs, 2H, NH_2); 4.18 (t, 2H, $J=5.0$ Hz, CH_2-5); 3.64 (s, 2H, CH_2-1′); 2.90 (t, 2H, $J=3.0$ Hz, CH_2-9); 1.90 (dt, 2H, $J=5.3$ Hz, C-7); 1.81 (dt, 2H, $J=5.2$ Hz, CH_2-6); 1.73 (dt, 2H, $J=5.3$ Hz, H-8). ^{13}C NMR ($CDCl_3$): δ 168.8 (C=O); 157.9 (C-2); 155.3 (C-10); 51.0 (C-5); 34.3 (C-1′); 30.1 (C-7); 27.3 (C-9), 27.2 (C-6); 24.7 (C-8). Anal. calcd for $C_9H_{15}N_5O$ (209.6): C, 51.66; H, 7.23; N, 33.47. Found: C, 51.43; H, 7.18; N, 33.29. *m/z* (EI) 209 $(M)^+$.

D-Galactose-[1-oxo-2-[6,7,8,9-tetrahydro-5*H*-[1,2,4]triazolo[1,5-*a*]azepin-2-yl]ethyl] hydrazone (14). A suspension of **13d** (0.8 g, 3.82 mmol) in EtOH (30 mL) and galactose (0.72 g, 4.0 mmol) was heated under reflux for 6 h. After cooling, the product was collected and recrystallized from EtOH to afford **14** (1.13 g, 80%) as an orange semi-solid. ^1H NMR (HMQC, DMSO-d_6/D_2O): δ 4.14 (pt, 2H, $J=5.0$ Hz, CH_2-5″); 3.78 (d, 1H, $J<1.0$ Hz, N=CH); 3.77 (m, 2H, H-4, H-5); 3.45 (ddd,

1H, $J_{5,6a}$ = 3.4 Hz, H-6a); 3.40 (s, 2H, CH_2-1′); 3.39 (m, 2H, H-2, H-3); 3.36 (dd, 1H, $J_{6a,6b}$ = 11.5 Hz, H-6b); 2.86 (t, 2H, J = 3.0 Hz, CH_2-9″); 1.80 (pt, 2H, J = 5.2 Hz, C-7″); 1.67 (pt, 2H, J = 5.2 Hz, CH_2-6″); 1.57 (pt, 2H, J = 5.4 Hz, H-8″); ^{13}C NMR (HMQC, DMSO-d_6/D_2O): δ 172.5 (C=O); 157.1 (C-2″); 155.5 (C-10″); 143.9 (N=CH); 76.5 (C-2); 73.4 (C-3); 70.0 (C-4); 68.3 (C-5); 60.4 (C-6); 50.0 (C-5″); 33.6 (C-1′); 29.4 (C-7″); 27.1 (C-9″), 26.4 (C-6″); 24.6 (C-8″). Anal. calcd for $C_{15}H_{25}N_5O_6$ (371.4): C, 48.51; H, 6.78; N, 18.86. Found: C, 48.32; H, 6.70; N, 18.64; m/z (FAB) 394 $(MNa)^+$.

2-[4-Phenyl-1*H*-1,2,4-triazole-5-thione-3-yl(methylene)]-6,7,8,9-tetrahydro-5*H*-[1,2,4]triazolo-[1,5-a]azepine (15). To a suspension of the hydrazide **13d** (0.42 g, 2.0 mmol), phenyl isothiocyanate (2.0 mmol) was added and the mixture was heated under reflux for 4 h. After cooling, the thiosemicarbazide solid was filtered, washed with EtOH, dried and used for the next step. The solid was dissolved in 5% NaOH solution (20 mL) and refluxed for 3 h. After cooling, the solution was treated with charcoal, filtered and acidified with dil. HCl to give a colorless solid. Recrystallization from EtOH afforded **15** (0.31 g, 50%) as a solid, mp 255–256 °C decomp. ^{1}H NMR (600 MHz, DMSO-d_6): δ 7.42 (m, 3H, Ar); 7.20 (m, 2H, Ar); 4.01 (t, 2H, J = 5.1 Hz, CH_2-5); 3.83 (s, 2H, CH_2-1′); 2.72 (t, 2H, J = 3.2 Hz, CH_2-9); 1.76 (m, 2H, C-7); 1.58 (m, 2H, CH_2-6); 1.50 (m, 2H, H-8). ^{13}C NMR (DMSO-d_6): δ 167.9 (C=S); 157.3 (C-2); 154.5 (C-10); 149.4 (C-3″); 133.5, 129.4, 129.1, 120.0, 128.6, 128.1 (Ar); 49.9 (C-5); 29.4 (C-1′); 26.9 (C-7); 26.2 (C-9), 25.3 (C-6); 24.5 (C-8). Anal. calcd for $C_{16}H_{18}N_6S$ (326.4): C, 58.87; H, 5.76; N, 25.75. Found: C, 58.65; H, 5.68; N, 25.62; m/z 326 $(M)^+$.

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