Novel 2,6-diketopiperazine-derived acetohydroxamic acids as promising anti-*Trypanosoma brucei* agents

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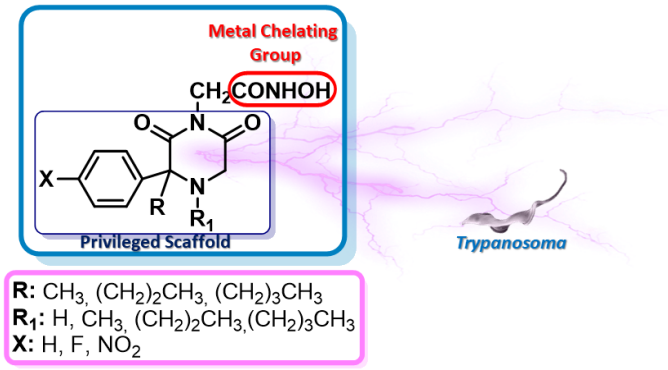
- **Aim:** Identification of new effective and selective trypanocidal agents. **Materials & methods:** Twelve novel acetohydroxamic acid derivatives based on 2-alkyl-2-aryl-2,6-diketopiperazine scaffolds have been synthesized and evaluated *in vitro* for their growth inhibitory activity against bloodstream form *T. brucei*. **Results:** All the analogues were remarkably potent inhibitors, with low micromolar to submicromolar activities. Structure-activity relationship studies demonstrated that the presence of an alkyl substituent at the *N*(4)-position of the 2,6-diketopiperazine ring portion was, in general, beneficial to trypanocidal activity in this series. **Conclusions:** The highest activity resulted from the introduction of a methyl, *n*-propyl or *n*-butyl substituent to the *N*(4)-position of the parent compound. Importantly, the most potent analogues were found to be highly selective against *T. brucei* with respect to mammalian cells.

- **Graphical abstract:** optional – if the authors wish, they can include a Figure to appear that is representative of their article.



- **Keywords:** 2-alkyl-2-aryl-2,6-diketopiperazine-1-acetohydroxamic acids, Anti-trypanosomal activity, Cytotoxicity on mammalian cells, NMR.

- **Main body of text:**

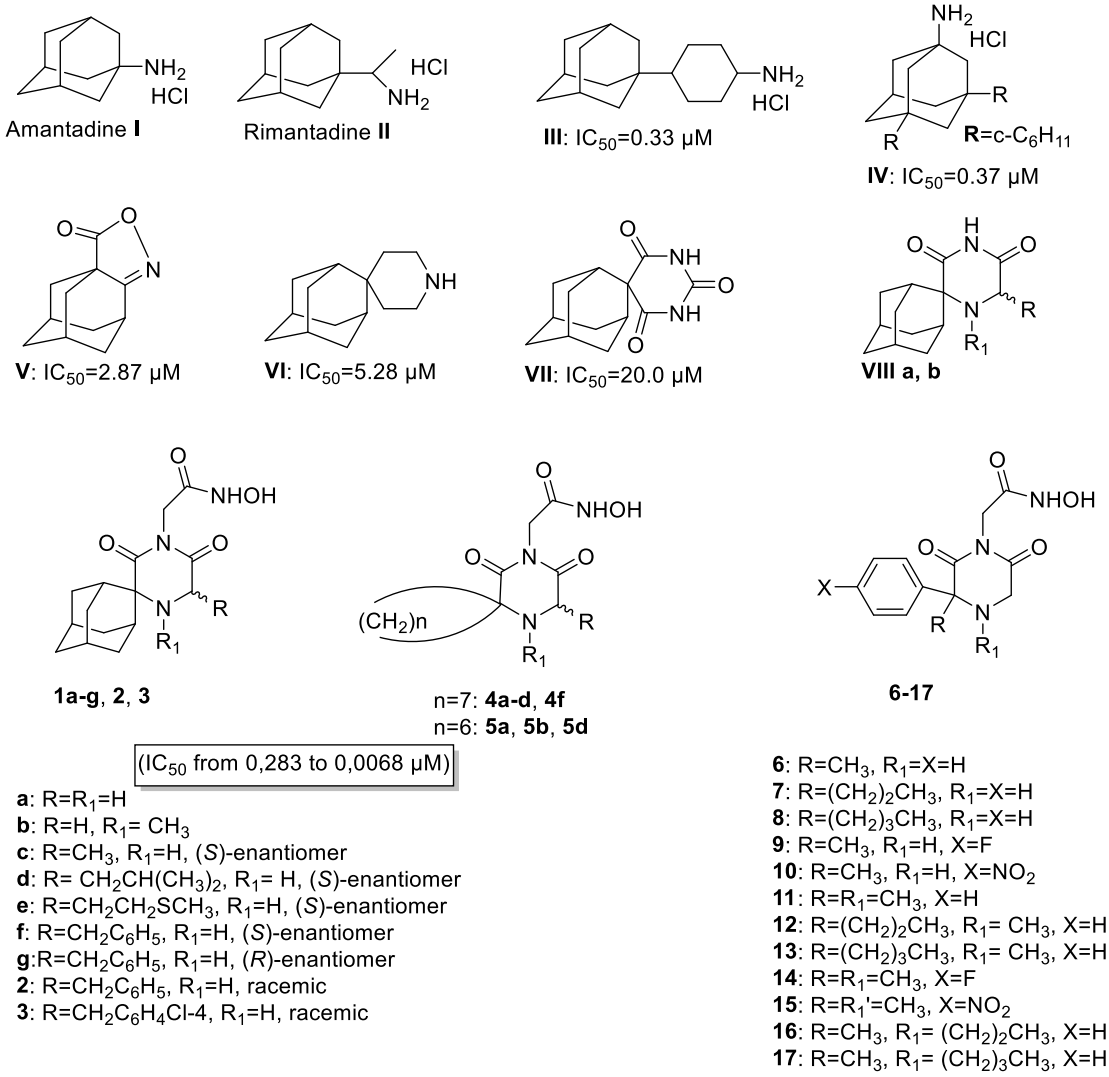
1. Introduction

Human African Trypanosomiasis (HAT) or sleeping sickness is amongst the most serious neglected tropical diseases and is caused by infection with parasitic protozoa of the *Trypanosoma brucei subspp* [1,2]. HAT constitutes a major public health risk within 36 sub-Saharan Africa countries due to its epidemic character [2,3]. It is estimated that 70 million people are at risk, and that around 3000 new infections occur every year in the endemic disease foci [2]. Current treatments for HAT have been based on old drugs including, suramin, pentamidine, melarsoprol and eflornithine, with an eflornithine-nifurtimox combination introduced in 2009 [1,2,4] although oral fexinidazole has shown considerable promise in clinical trial and has recently been recommended for use ([5] Ku Mesu, V.K.B.K., Kalonji, W.M., Bardonneau, C., Mordt, O.V., Blesson, S. (2018) Oral fexinidazole for late-stage African Trypanosoma brucei gambiense trypanosomiasis: a pivotal multicentre, randomised, non-inferiority trial. Lancet 391, 144-154. [6] European Medicines Agency recommends fexinidazole, the first all-oral treatment for sleeping sickness. https://www.dndi.org/2018/media-centre/press-releases/). These drugs are often associated with severe toxic side effects, poor efficacy, and problematic administration. Additionally, the HAT drugs are expensive, and their usage requires adequate medical care, which is not readily available in the most affected regions of sub-Saharan Africa [2,4]. All the above issues with the existing HAT drugs emphasize an imperative need for research and development of new efficient, safe, and affordable antitrypanosomal therapeutics.

In 1999, it was discovered that bloodstream form *T. brucei* are sensitive *in vitro* to the anti-influenza A drugs amantadine **I** and rimantadine **II**. Rimantadine was also found to be toxic to the trypanosomatid parasites *T. cruzi* and *Leishmania major*.[5] Two years later, it was reported that a series of aminoadamantane and aminoalkylcyclohexane derivatives are effective growth inhibitors of *T. brucei in vitro* and *in vivo*, and that inhibition was correlated with the hydrophobicity of the compounds. Some of these derivatives (**III**-**IV**, Figure 1) showed submicromolar trypanocidal activities *in vitro*; in particular the adamantane analogue **III** (IC_{50}=0.33 μM) gave 400- and 21-fold increases in antitrypanosome potency compared to amantadine and rimantadine, respectively.[6] In our earlier works we communicated the trypanocidal properties of some nitrogen-containing adamantane derivatives (amines or not).[7,8] Among them, compounds **V-VII** (Figure 1) possessed considerable activities *in vitro* against *T. brucei*. Oxazolone **V**[8] was the most active inhibitor of the parasite growth, exhibiting a potency that was 3-fold higher than rimantadine and at least 45-fold greater than amantadine, while the trypanocidal activity of spiro piperidine **VI**[7] was found to be 1.5 times more than rimantadine, and at least 25 times greater than amantadine. The spiro barbituric analogue **VII**[7] displayed more potent inhibition (7-fold) than that of amantadine, although it was ~3-fold less effective than rimantadine.

In pursuit of a better antitrypanosome potency we explored the trypanocidal properties of the structurally related spiro 2,6-diketopiperazine derivatives **VIIIa** and **VIIIb**. Unfortunately, these compounds were only marginally active against *T. brucei* parasite. Yet, compounds **VIIIa** and **VIIIb** as well as other lipophilic spiro carbocyclic 2,6-DKPs represent useful scaffolds that can be transformed into potent trypanocidal agents, with single nanomolar to submicromolar activities, by introducing an acetohydroxamic acid moiety to their imidic nitrogen [5,6]. Thus, we produced a series of lipophilic, constrained spiro carbocyclic 2,6-diketopiperazine-1-acetohydroxamic acid derivatives (Fig. 1., **1a-g**, **2**, **3**, **4a-d**, **4f**, **5a**, **5b**, **5d**) that displayed single nanomolar to submicromolar activities. [5,6] SAR studies showed the indispensability of the hydroxamic unit (CONHOH) for the trypanocidal activity in this class of compound [5]. Thus, we presumed that these hydroxamates act by inhibiting a decisive parasite metalloenzyme due to the metal ion coordinating properties exerted by the hydroxamic acid group in the catalytic site. We have also confirmed that incorporating a benzyl rather than an aliphatic substituent into the methylene carbon next to the basic nitrogen of the spiro carbocyclic 2,6-DKP portion leads to analogues (Fig. 1., **1f**, **1g**, **2**, **3** and **4f**) with the higher trypanocidal activity.

In order to identify the structural features of the 2,6-DKP-based acetohydroxamic acids required for potent trypanocidal activity, we modified the spiro carbocyclic 2,6-DKP core structure by changing the spiro-linked carbocycle component for an alkyl and an aryl substituent. In this report, we present the design and synthesis of a new series of acetohydroxamic acid analogues (Fig. 1. **6**-**17**) as *T. brucei* growth inhibitors based on conformationally non-constrained 3-alkyl-3-aryl-2,6-DKP scaffolds. Within this series, we studied the trypanocidal potency of compounds in relation to: (a) the length of the C-3 *n*-alkyl substituent (compounds **6**-**8**), (b) the *para*-substitution on the aromatic ring with fluorine atom or nitro group (compounds **9** and **10**), and (c) the alkyl substitution at the *N*(4)-position of the 2,6-DKP ring (compounds **11**-**17**). The antitrypanosomal properties of these novel compounds were assessed against *T. brucei* bloodstream form parasites *in vitro*.



(IC_{50} from 0,283 to 0,0068 μM)

a: R=R_1=H
b: R=H, R_1= CH_3
c: R=CH_3, R_1=H, (*S*)-enantiomer
d: R= $CH_2CH(CH_3)_2$, R_1= H, (*S*)-enantiomer
e: R=$CH_2CH_2SCH_3$, R_1=H, (*S*)-enantiomer
f: R=$CH_2C_6H_5$, R_1=H, (*S*)-enantiomer
g:R=$CH_2C_6H_5$, R_1=H, (*R*)-enantiomer
2: R=$CH_2C_6H_5$, R_1=H, racemic
3: R=$CH_2C_6H_4Cl$-4, R_1=H, racemic

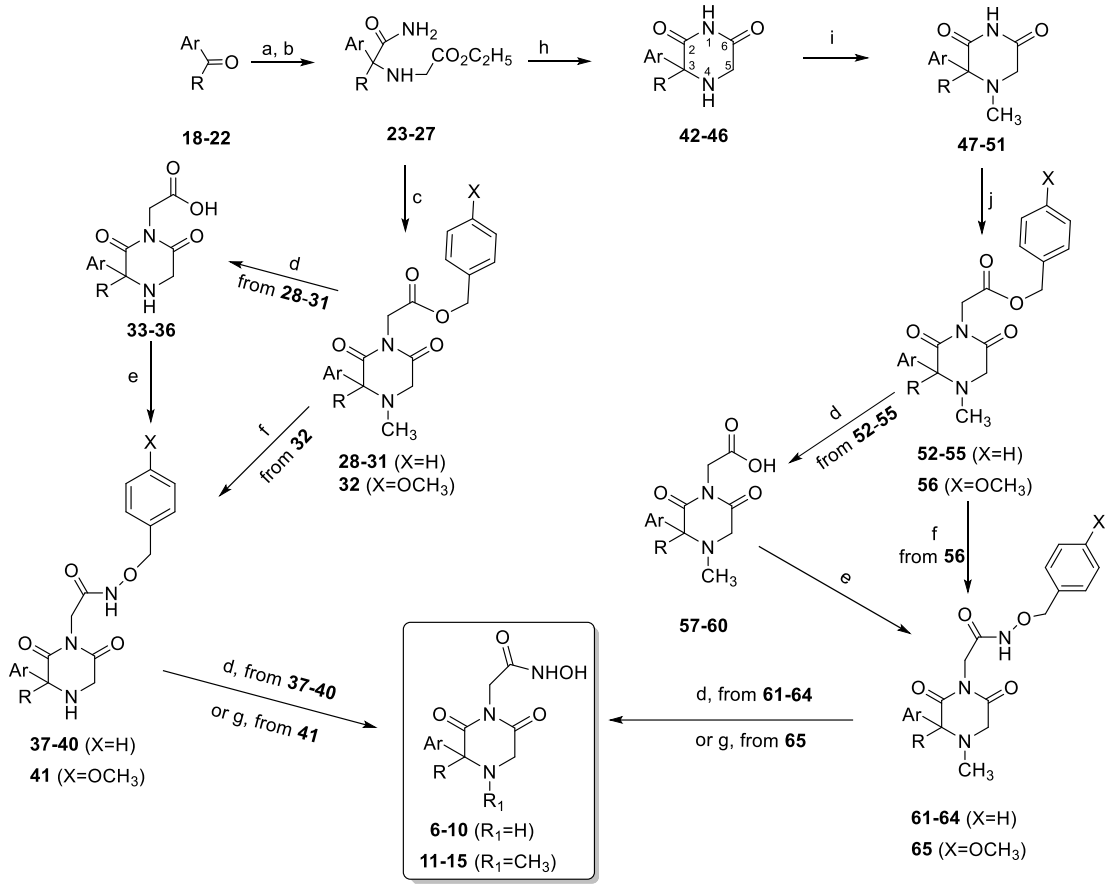
6: R=CH_3, R_1=X=H
7: R=$(CH_2)_2CH_3$, R_1=X=H
8: R=$(CH_2)_3CH_3$, R_1=X=H
9: R=CH_3, R_1=H, X=F
10: R=CH_3, R_1=H, X=NO_2
11: R=R_1=CH_3, X=H
12: R=$(CH_2)_2CH_3$, R_1= CH_3, X=H
13: R=$(CH_2)_3CH_3$, R_1= CH_3, X=H
14: R=R_1=CH_3, X=F
15: R=R_1'=CH_3, X=NO_2
16: R=CH_3, R_1= $(CH_2)_2CH_3$, X=H
17: R=CH_3, R_1= $(CH_2)_3CH_3$, X=H

Figure 1. Structures of amantadine **I**, rimantadine **II**, lipophilic adamantane derivatives **III**-**VII**, 2,6-DKPs **VIIIa** and **VIIIb**, spiro carbocyclic 2,6-diketopiperazine-1-acetohydroxamic acid derivatives **1a-g**, **2**, **3**, **4a-d**, **4f**, **5a**, **5b**, **5d** reported previously [X-X], and structures of the new acetohydroxamic acid analogues **6**-**17** based on 3-alkyl-3-aryl-2,6-diketopiperazine scaffolds.

2. Results and Discussion

2.1. Chemistry

Compounds **6**-**17** were synthesized following similar procedures to those reported in our previous publications (Schemes 1 and 2) [5-7]. As shown in Scheme 1, the Strecker reaction of the ketones **18**-**22** with ethyl glycinate hydrochloride and sodium cyanide, and subsequent acidic hydration (H_2SO_4 97%) of the unstable α-aminonitrile intermediates (not shown) provided the respective amide-ester derivatives **23**-**27**, which served as key compounds for further elaboration. Treatment of compounds **23**-**27** with potassium bis(trimethylsilyl)amide in THF gave, after an SN2 reaction of the intermediate potassium imidate salts with benzyl or 4-methoxybenzyl bromoacetate in DMF, the corresponding 2,6-DKP-1-acetic acid benzyl ester derivatives **28**-**32**. An analogous base-catalyzed intramolecular cyclization of the amide-ester derivatives **23**-**27** using potassium bis(trimethylsilyl)amide (1eq), followed by the addition of TFA (1eq) led to their respective 2,6-DKPs **42**-**46**. Reductive methylation on the basic nitrogen atom of the 2,6-DKPs **42**-**46** with $CH_2O/NaCNBH_3$ in MeOH or MeOH-THF 1:1 gave the corresponding methyl substituted analogues **47**-**51**. The latter compounds, upon reaction with benzyl or 4-methoxybenzyl bromoacetate in the presence of sodium hydride in DMF, were converted to the N-methylated 2,6-DKP-1-acetic acid benzylester derivatives **52**-**56**. Catalytic hydrogenolysis (H_2/10% Pd-C) of the benzyl esters **28**-**31** and **52**-**55** occurred cleanly to afford the carboxylic acids **33**-**36** and **57**-**60**, which underwent efficient CDI coupling reactions with *O*-benzylhydroxylamine to give the *O*-benzyl hydroxamates **37**-**40** and **61**-**64**, respectively. The desired hydroxamic acids **6**-**9** and **11**-**14** were available via catalytic hydrogenolysis (H_2/10% Pd-C) of the benzyl-protected hydroxamates **37**-**40** and **61**-**64**, respectively. Additionally, treatment of the 4-methoxybenzyl esters **32** and **56** with TFA, followed by CDI-catalyzed coupling reactions of the respective carboxylic acid intermediates (not shown) with *O*-(4-methoxybenzyl)hydroxylamine gave the corresponding *O*-(4-methoxybenzyl) hydroxamates **41** and **65**. The removal of the 4-methoxybenzyl protecting group of **41** and **65** was achieved by exposure to TFA in the presence of triethylsilane in CH_2Cl_2 affording the targeted nitro-substituted hydroxamic acid analogues **10** and **15**, respectively.

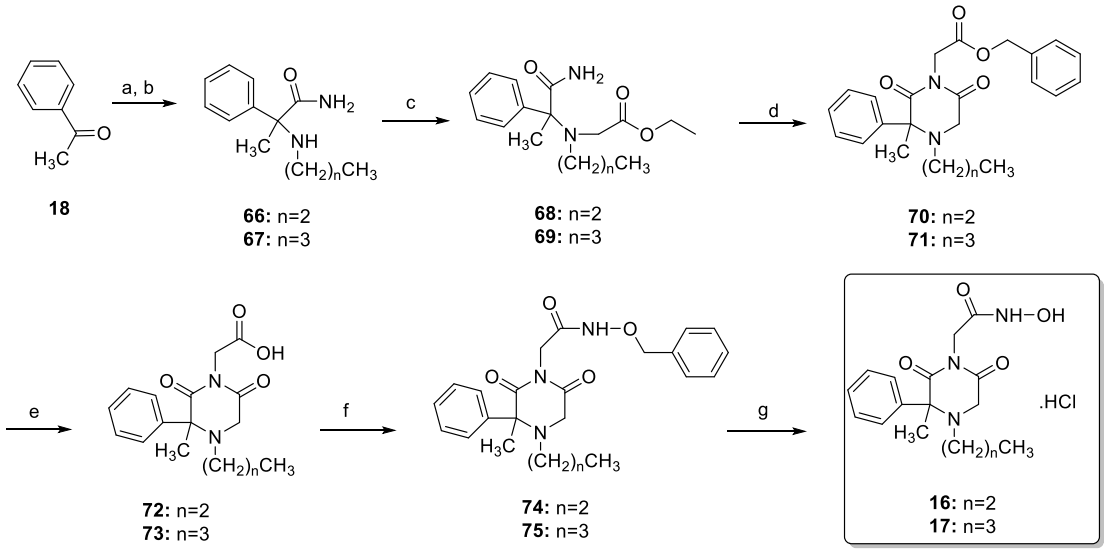


18, 23, 28, 33, 37, 6, 42, 47, 52, 57, 61, 11:	R= CH_3, Ar= C_6H_5
19, 24, 29, 34, 38, 7, 43, 48, 53, 58, 62, 12:	R= $(CH_2)_2CH_3$, Ar= C_6H_5
20, 25, 30, 35, 39, 8, 44, 49, 54, 59, 63, 13:	R= $(CH_2)_3CH_3$, Ar= C_6H_5
21, 26, 31, 36, 40, 9, 45, 50, 55, 60, 64, 14:	R= CH_3, Ar= 4-FC_6H_4
22, 27, 32, 41,10, 46, 51, 56, 65, 15:	R= CH_3, Ar= 4-$NO_2C_6H_4$

Scheme 1. Reagents and conditions: **(a)** NaCN, $H_2NCH_2CO_2Et.HCl$, DMSO/H_2O 29:1 (v/v), rt, 48h; **(b) (i)** H_2SO_4 97%, CH_2Cl_2, rt, 24h; **(ii)** ice and then aq. NH_3 26% to pH 7-8, 20-55% yields over two steps; **(c) (i)** $(Me_3Si)_2NK$ (1 eq), THF, 0-5°C then rt, 2h, argon; **(ii)** $BrCH_2CO_2CH_2C_6H_5$ or $BrCH_2CO_2CH_2C_6H_5OCH_3$-4 only for **32**, DMF, rt, 48h, argon, 73-91%; **(d)** H_2/Pd-C, EtOH or EtOH-AcOEt 3:1 for **35**, 50 psi, rt, 3h, 96- >99% for **33**-**36**, **57**-**60**, 75-94% for **6-9**, **11-14**; **(e) (i)** CDI, THF for **37**-**40, 61-63** or THF-DMF 4:1 for **64**, 28 °C for **37**-**39, 61**-**63** or 55 °C for **40**, **64**, 1h, argon; (ii) $C_6H_5CH_2ONH_2.HCl$, Et_3N, 28 °C, 24h and then 45 °C, 1h, argon, for **37**-**39**, **61**-**63** or 55 °C, 25h, argon, for **40**, **64**, 61-76%; **(f) (i)** CF_3CO_2H, CH_2Cl_2, rt, 90 min; **(ii)** Et_3N, CDI, THF, 28 °C, 1h, argon; **(iii)** 4-$CH_3OC_6H_4CH_2ONH_2$, 28 °C, 18h, then 55 °C, 7h, argon, 48% and 43% yields over two steps for **41** (from **32**) and **65** (from **56**), respectively; **(g)** CF_3CO_2H, CH_2Cl_2, rt, 10 min, then Et_3SiH, rt, 45 min, 63% for **10** (as hydrochloride) from **41**, 70% for **15** from **65**; (h) as (c) (i), then CF_3CO_2H (1 eq) 91-96%; (i)(i) aq CH_2O 37%, MeOH or MeOH-THF 1:1 for **51**, rt, 3h, then $NaCNBH_3$, rt, 4h at pH 6-7 (maintaining by adding AcOH); (ii) NaOH 1N and Na_2CO_3 to pH 8, 74-88%; (j) NaH, DMF, rt, 1h or 10 min for **56**, argon and then as (c) (ii) using $BrCH_2CO_2CH_2C_6H_5$ or $BrCH_2CO_2CH_2C_6H_5OCH_3$-4 only for **56**, 71-87%.

Scheme 2 shows the synthesis of the hydroxamic acid analogues **16** and **17**, bearing a *n*-propyl (**16**) or *n*-butyl (**17**) aliphatic substituent at the basic nitrogen atom of the 2,6-DKP scaffold. Treatment of acetophenone **18** with *n*-propylamine or *n*-butylamine hydrochloride and sodium cyanide, followed by acid-catalyzed hydration of the unstable *α*-aminonitrile intermediates (not shown) gave the respective amino amides **66** and **67**. These

compounds were then reacted with ethyl bromoacetate in the presence of sodium bicarbonate in DMF to provide the corresponding amide-ester derivatives **68** and **69**. Employing a four step reaction sequence similar to that described above for the preparation of the hydroxamic acid congeners **6**-**9**, the amide-ester derivatives **68** and **69** were converted to the hydroxamic acid analogues **16** and **17**, respectively. The ^{1}H and ^{13}C NMR spectra for the acetohydroxamic acid analogues described in this report (compounds **6**-**17**) are consistent with E/Z conformational behavior of these molecules in solution. The assignment of the E and Z isomers was based on our E/Z conformational isomerism study reported previously [8].



Scheme 2. Reagents and conditions: **(a)** NaCN, $CH_3(CH_2)_2NH_2.HCl$ for **66** ή $CH_3(CH_2)_3NH_2.HCl$ for **67**, DMSO/H_2O 29:1, rt, 48h; **(b) (i)** H_2SO_4 97%, CH_2Cl_2, rt, 24h, **(ii)** ice and then aq. NH_3 26% to pH 7-8, 45% (**66**) and 47% (**67**) yields over two steps; **(c)** $BrCH_2CO_2Et$, $NaHCO_3$, DMF**,** 40-43 °C, 6d, 57% for **68**, 47% for **69**; **(d) (i)** $(Me_3Si)_2NK$, THF, 0-5 °C and then rt, 2h, argon; **(ii)** $BrCH_2CO_2CH_2C_6H_5$, DMF, rt, 48h, argon, 89% for **70**, 81% for **71**; **(e)** H_2/Pd-C, EtOH, 50 psi, rt, 3h, 93% for **72**, 98% for **73**; **(f) (i)** CDI, THF, 28 °C, 1h, argon, **(ii)** $C_6H_5CH_2ONH_2.HCl$, Et_3N, 28 °C, 24h and then 45 °C, 1h, argon, 65% for **74** and **75**; **(g) (i)** as (**e**), **(ii)** HCl in Et_2O, 70% for **16,** 68% for **17**.

2.2. Biological Activity

The newly synthesized hydroxamic acid derivatives **6**-**17** were tested against bloodstream form *T. brucei in vitro*. The IC_{50} and IC_{90} values for each compound are summarized in Table 1. As shown, 10 out of the 12 tested compounds had IC50s values in the low to submicromolar range against *T. brucei* (compounds **6**-**8** and **11**-**17**) in the free base and hydrochloride forms. The cytotoxicities of the most active compounds against mammalian cells were also determined using the rat skeletal myoblast L6 cells (Table 1.), with most displaying very favorable selective indices.

The initial compound prepared in this 3-alkyl-3-aryl-2,6-DKP-1-acetohydroxamic acid series, **6**, exhibited appreciable trypanocidal activity both as free base and hydrochloride salt, with IC_{50}s of 6.97 and 6.61 μM, respectively. Replacement of the C-3 methyl substituent in the 2,6-DKP sacaffold of **6** with *n*-propyl or *n*-butyl side chains led to the respective more lipophilic C-3 alkyl substituted analogues **7** and **8**. These compounds displayed activities that were comparable to that of **6**; the C-3 propyl analogue **7** (IC_{50}=7.25 μM or 6.93 μM as hydrochloride) was almost equipotent to the parent structure **6**, whereas the C-3 butyl counterpart **8** (IC_{50}=1.72 or 1.85 μM as hydrochloride) had approximately 4-fold higher activity than **6** and **7**. However, it is apparent that lengthening of the C-3 alkyl chain in compounds **7** and **6** by one and three methylene carbons, respectively, boosted potency towards *T. brucei* to a noteworthy level (**8** vs **6** and **7**). These results demonstrate that the lipophilicity and / or possible steric effects of the C-3 n-alkyl chain influence the trypanocidal activity in this subset of compounds.

Substitution at *para*-position of the phenyl moiety in the parent structure **6** by either a lipophilic or hydrophilic electron-withdrawing substituent, such as a fluorine atom or a nitro group was slightly detrimental to activity. The *p*-fluoro substituted analogue **9** was 2.7 and 2 times less potent than the parent **6**, when these compounds were tested in the free base and hydrochloride salt forms, respectively, while the *p*-nitro congener **10** proved 1.8-fold less potent than **6** when comparing the IC_{50}s of their corresponding hydrochloride salts. On the other hand, the *para*-fluoro analogue **9** and the *para*-nitro congener **10** displayed almost equal activity in the form of their corresponding hydrochloride salts (**9**, IC_{50}=12.9 μM; **10**, IC_{50}=11.7 μM). These findings imply that the observed activity-decrease in compounds **9** and **10** was largely unaffected by the lipophilic or hydrophilic properties, as well as the size of the electron-withdrawing *para*-substituent.

The addition of a methyl substituent to the N(4)-position of the 2,6-DKP ring [N(4)-methylation] in the parent compounds **6**, **7**, **9** and **10** appeared to have a favorable effect on the trypanocidal activity and resulted in a 1.5-12-fold increase in potency for the respective *N*-methylated analogues **11**, **12**, **14** and **15**. The greatest improvement in potency was observed with the *N*-methyl analogue **11** (IC_{50}=0.59 μM or 0.55 μM as hydrochloride), which showed a significantly increased trypanocidal activity (12-fold), relative to the unsubstituted parent **6**. The *N*-methyl derivatives **12** and **15** also gave quite better potencies compared to the corresponding parent structures **7** and **10**; the C-3 propylated free base **12** and its hydrochloride (**12.HCl**) had 4.1 and 5.5 times greater trypanocidal activity than **7** and **7.HCl**, respectively, whereas compound **15** in the form of its hydrochloride salt (**15.HCl**) was 9-fold more potent than the hydrochloride salt of **10**. However, a slight decrease (1.5-fold) in activity was detected in the case of the C-3 butylated *N*-methyl counterpart **13** upon *N*(4)-methylation of the corresponding parent molecule **8**. It is interesting that the effect of the *N*(4)-methylation on the trypanocidal activity within the *N*-methyl derivatives series **11**-**13** seemed to be inversely related to the length of the alkyl side chain at the 3-position of the 2,6-DKP ring, as observed by comparing the analogue pairs **11**/**6**, **12**/**7** and **13**/**8**. While the N(4)-methylation of the parent structure **6** led to a significant increase in potency (12-fold) for the C-3 methyl-substituted *N*-methyl analogue **11**, it was less beneficial (approximately 4 to 5-fold increase) and slightly detrimental (1.5-fold decrease) in the cases of the C-3 propyl- and C-3 butyl-substituted *N*-methyl counterparts **12** and **13**, respectively. These results might be due to the steric interference in the active site.

The marked difference in increasing potency between the *N*-methylated derivative **11** and the corresponding *NH*-analogue **6** prompted the introduction of a longer hydrophobic alkyl substituent such as *n*-propyl or *n*-butyl groups to the N(4)-position of the 2,6-DKP scaffold in parent **6**. These *N*(4)-alkyl substitutions led to significantly effective derivatives which inhibited *T. brucei* growth at submicromolar concentrations. The *N*-propyl derivative **16** (IC_{50}=0.47 μM) and the *n*-butyl counterpart **17** (IC_{50}=0.63 μM) in the form of their hydrochloride salts proved 14- and 10.5-fold more potent than the hydrochloride salt of the *N*-alkyl free congener **6**, respectively. However, both *N*-alkylated compounds **16** and **17** exhibited trypanocidal potencies similar (in the order of 0.5 μM) to the potency of the *N*-methyl derivative **11**. These results indicate that the length and lipophilicity of the N(4)-alkyl substituent were not important for potent activity within the group of the acetohydroxamic acid analogues **11**, **16**, and **17**, in which the C-3 alkyl substituent of the 2,6-DKP scaffold is identical.

Notably, the most active compounds in this series (**8**, **11**-**13** and **15**-**17**) displayed remarkably low cytotoxicity against mammalian cells (with the exception of compounds **8** and **13**), having selectivity indices ranging from 180 (**12**) to 1180 (**11**). In particular, the acetohydroxamic acid derivatives **11**, **16** and **17,** which had the highest activity against *T. brucei* (IC_{50}=0.55, 0.47 and 0.63 μM, respectively), displayed the best selectivity with respect to

L6 cells.

Table 1. Activity of acetohydroxamic acid analogues **6-17** tested against cultured bloodstream-form *T. brucei* (pH=7.4) and cytotoxicity of the most active compounds against cultured rat skeletal myoblast L6 cells.

	Activity	

Cpds	*T. brucei*		Cytotoxicity L6 cells	
	IC_{50} (μM)[a,b,e]	IC_{90} (μM)[a,b,e]	IC_{50} (μM)[c,e]	SI[d,e]
6	6.97±1.37 (6.61±1.65)	19.86±2.99 (17.2±1.78)	ND	-
7	7.25±0.25 (6.93±0.26)	10.4±0.5 (8.77±0.23)	ND	-
8	1.72±0.38 (1.85±0.17)	7.54±0.72 (6.32±0.65)	ND (39±2)	(21)
9	19.1±2.5 (12.9±0.6)	32.9±1.2 (20.3±0.5)	ND	-
10	(11.7±3.6)	(23.2±2.5)	ND	-
11	0.59±0.1 (0.55±0.06)	1.33±0.17 (1.35±0.12)	698±69 (549±40)	1180 (1000)
12	1.77±0.05 (1.27±0.1)	2.23±0.02 (1.95±0.06)	322±25 (315±51)	180 (248)
13	2.65±0.25 (2.97±0.38)	4.71±0.09 (6.18±1.11)	24±1 (ND)	9
14	8.16±0.59 (7.86±0.58)	14.5±0.32 (13.6±0.38)	ND	-
15	1.22±0.24 (1.29±0.16)	2.89±0.24 (2.14±0.19)	373±18 (397±21)	305 (310)
16[d]	(0.47±0.02)	(1.13±0.15)	(354±37)	(750)
17[d]	(0.63±0.08)	(1.39±0.03)	(186±32)	(295)

[a]Concentrations required to inhibit growth of *T. brucei* by 50% and 90%, respectively.

[b]IC_{50} and IC_{90} data are the mean of triplicate experiments ± SEM.

[c]Cytotoxicity was determined by establishing the concentration required to inhibit growth of cultured L6 cells by 50% (IC_{50}). Data are the mean of triplicate experiments ± SEM.

[d]Selectivity indices were calculated as the ratio of the IC_{50} for L6 cells and *T. brucei.*

[e]Data in brackets refer to the respective hydrochloride. ND: Not determined.

3. Conclusion

We have developed a novel series of acetohydroxamic acid derivatives that inhibit the bloodstream form *T. brucei* parasite growth with low micromolar or submicromolar IC_{50} values. These inhibitors were derived from 3-alkyl-3-aryl-2,6-DKP scaffolds by incorporating an acetohydroxamic acid moiety as metal chelating group in their imidic nitrogen atom. Nevertheless, the new class of compounds were found to be less potent than the spiro carbocyclic 2,6-DKP congeners **1**-**5**.[x,x] The observed decrease in antitrypanosome potency of the new compounds **6**-**17** might be ascribe to their lower lipophilicity, and unfavorable stereoelectronic factors. Within the *N*(4)-alkyl free analogues **6**-**8**, a C-3 propyl instead of C-3 methyl substitution resulted in an almost equivalent antitrypanosome effect (compare **7** to **6**), whereas a significant enhancement in potency was observed upon C-3 butyl substitution (**8** *vs* **6**). Substitution at the *para*-position of the phenyl moiety in the parent **6** by a fluorine atom or nitro group resulted in a slight reduction of activity (compound **9** and **10**).

Introduction of a methyl substituent to the *NH*-position of the 2,6-DKP ring [*N*(4)-methylation] in the unsubstituted compounds **6**-**10** has, in general, a positive influence on the potency against *T. brucei*, as represented by the *N*(4)-methyl analogues **11**, **12**, **14** and **15**. Among the latter compounds, the *N*(4)-methyl derivative **11** was the most potent against trypanosomes, with a submicromolar IC_{50} value (0.55 μM). However, a similar submicromolar *T. brucei* inhibitory effect (approximately 0.5 μM) was obtained, when the *N*(4)-methyl group in **11** was replaced by a propyl or butyl *n*-alkyl chain, as in the respective *N*(4)-substituted counterparts **16** and **17** (Table 1.). Importantly, the most potent compounds of this series were found to be highly selective in inhibiting *T. brucei* growth over mammalian cells. Currently, the target of these acetohydroxamic acid derivatives in trypanosomes is unknown. Their submolar potency against *T. brucei* and their relative lack of toxicity to mammalian cells suggests that general metal chelation activity is unlikely. Rather, we would favor a more specific mode of action, analogous to inhibitors of histone deacetylase that have anti-cancer potential, where the mechanism involves binding of the hydroxamate to zinc ions in the catalytic site of the enzyme **(**Finnin MS, Donigian JR, Cohen A, Richon VM, Rifkind RA, Marks PA, Breslow R, Pavletich NP (1999) Structures of a histone deacetylase homologue bound to the TSA and SAHA inhibitors. Nature 401, 188–193.). Identification of a target in trypanosomes would greatly aid the design of more effective inhibitors.

4. Materials & Methods

4.1 Chemistry

4.1.1. General

Melting points were determined using a Büchi capillary apparatus and are uncorrected. 1H and 13C NMR spectra were obtained on Bruker MSL 400 (400 MHz ^{1}H, 100 MHz ^{13}C), Bruker AVANCE III 600 (600 MHz ^{1}H, 150 MHz ^{13}C) and Bruker AVANCE 200 (50 MHz ^{13}C) spectrometers, using $CDCl_3$ or DMSO-d_6 as solvent. Chemical shifts are reported in δ(ppm) with tetramethylsilane or solvent (DMSO-d_6) as internal standard. Splitting patterns are designated as s, singlet; d, doublet; dd, doublet of doublets; t, triplet; td, triplet of doublets; q, quartet; qd, quartet of doublets; m, multiplet; dm, doublet of multiplets; br, broad; v br, very broad; sym, symmetrical. The spectra were recorded at 293 K (20 °C) unless otherwise specified. Carbon multiplicities were established by DEPT experiments. 2D NMR experiments (HMQC and COSY) were performed for the elucidation of the structures of the newly synthesized compounds. Low-resolution mass spectra were recorded on either an API 2000 LC-MS/MS or Thermo-Finnigan AQA model LC-MS system, using positive electrospray ionization mode or Thermo Electron Corporation DSQ mass spectrometer in chemical ionization (CI) in positive ion mode with methane as CI reagent gas or in electron impact (EI). High-resolution mass spectra (HRMS) were determined on a hybrid LTQ-Orbitrap Discovery mass spectrometer under electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI) in positive ion mode. Analytical thin-layer chromatography (TLC) was conducted on precoated Merck silica gel 60 F_{254} plates (layer thickness 0.2 mm) with the spots visualized by iodine vapors and/or UV light. Column chromatography purification was carried out on silica gel 60 (70-230 and 230-400 mesh ASTM). Elemental analyses (C, H, N) were performed by the Service Central de Microanalyse at CNRS (France) or Department of Microanalysis of NCSR "Democritos" (Greece), and were within ±0.4% of the calculated values, except where noted (compounds **10** and **33.HCl**). The purities of the tested compounds were determined by analytical HPLC and elemental analysis. The obtained results correspond to >95% purity. Analytical HPLC was performed on a Thermo Finnigan HPLC system (Thermo Finnigan, San Jose, USA) consisting of a SpectraSystem P4000 pump, a SpectraSystem 100 degasser, a SpectraSystem AS3000 autosampler, and a SpectraSystem UV2000 PDA detector, controlled by a SpectraSystem controller. ChromQuest 4.1 software was used for the management of the data. For the HPLC-DAD, a Supelco Analytical Discovery HS C18 (250 mm x 4.6 mm, 5.0 μm)

column was used and the injection volume was 10 µL. The mobile phase consisted of H_2O and 1% acetic acid (solvent A) and acetonitrile (solvent B), and solvent gradient of A/B was 95/5 to 0/100. The analyses were performed at r.t. with a constant flow rate of 1 mL/min using a gradient elution of 0-50 min. The commercial reagents were purchased from Alfa Aesar, Sigma-Aldrich and Merck, and were used without further purification except for the benzyl bromoacetate and ethyl bromoacetate. These reagents were purified by distillation prior to use. 4-Methoxybenzyl bromoacetate used, was prepared according to our previously published experimental protocol [6]. *O*-(4-Methoxybenzyl)hydroxylamine was synthesized according to the literature reported method [9]. Organic solvents used were in the highest purity, and when necessary, were dried by the standard methods. Yields refer to chromatographically pure materials.

4.1.2. N-[(1-Aminocarbonyl-1-phenyl)ethyl]glycine ethyl ester **23**

To a stirred suspension of sodium cyanide (1.37 g, 27.9 mmol) and ethyl glycinate hydrochloride (3.9 g, 27.9 mmol) in 18 mL of DMSO-H_2O 9:1 (v/v), a solution of acetophenone **18** (3.2 g, 26.6 mmol) in DMSO (36 mL) was added in one portion. The reaction flask was sealed, and the mixture was allowed to react for 48 h at room temperature with stirring. After this time, the mixture was poured into ice-water (200 mL) and extracted with Et_2O (4 × 60 mL). The combined organic extracts were washed with water (2 × 50 mL), dried, and the solvent was evaporated in vacuo at room temperature to avoid thermal decomposition of the reaction product (*α*-aminonitrile). The obtained oily residue was dissolved in CH_2Cl_2 (140 mL), cooled in an ice-water bath, and treated dropwise with H_2SO_4 97% (32 mL) under vigorous stirring. The resulting mixture was stirred at room temperature for 24 h, and carefully poured into crashed ice (170 g). To this two-phase mixture, aqueous NH_3 26% was added dropwise to pH=7-8 with stirring under cooling (ice-water). The phases were separated, and the aqueous layer was extracted with CH_2Cl_2 (3 × 100 mL). The combined organic fractions were washed once with water (100 mL) and once with brine (100 mL), dried, and evaporated in vacuo. The remaining crude oil was purified by flash column chromatography with AcOEt-Et_2O 1:1, as eluent, to afford the title compound **23** as a white crystalline solid (1.35 g, 20% overall yield): mp 88-90 °C (AcOEt/Et_2O-*n*-pentane); ^{1}H NMR (400 MHz, $CDCl_3$) *δ* 1.15 (t, 3H, *J*=7.1 Hz, $CO_2CH_2CH_3$), 1.62 (s, 3H, CH_3), 2.26 (s, 1H, $NHCH_2CO_2CH_2CH_3$), 3.11-3.26 (~q, 2H, *J*=17.3 Hz, $NHCH_2CO_2CH_2CH_3$), 4.0-4.14 (q, 2H, *J*=7.1 Hz, $CO_2CH_2CH_3$), 6.43 (br s, 1H, CONHH), 6.94 (br s, 1H, CONHH), 7.15-7.51 (m, 5H, aromatic H); ^{13}C NMR (100 MHz, $CDCl_3$) *δ* 14.0 ($CO_2CH_2CH_3$), 22.6 (CH_3), 45.4 ($NHCH_2CO_2CH_2CH_3$), 60.9 ($CO_2CH_2CH_3$), 64.5 ($C_6H_5(CH_3)CNH$), 126.2, 127.5, 128.4 (2,3,4,5,6-aromatic C), 141.6 (1-aromatic C), 172.1 ($CO_2CH_2CH_3$), 177.5 ($CONH_2$). Anal. Calcd for $C_{13}H_{18}N_2O_3$: C, 62.38; H, 7.25; N, 11.19; Found: C, 62.24; H, 7.10; N, 11.24.

4.1.3. N-[(1-Aminocarbonyl-1-phenyl)butyl]glycine ethyl ester **24**

Prepared from butyrophenone **19** (3.95 g, 26.6 mmol) according to the method described for compound **23**. The crude oil was purified by column chromatography on silica gel with AcOEt-*n*-hexane 1:1, as eluent, to afford the title compound **24** as a colorless viscous oil (1.5 g, 20% overall yield); ^{1}H NMR (400 MHz, $CDCl_3$) *δ* 0.87 (t, 3H, *J*=7.3 Hz, $CH_3(CH_2)_2$), 1.06-1.21 (complex m, 5H, $CH_3CH_2CH_2$, $CO_2CH_2CH_3$), 1.94-2.18 (dm, 2H, $CH_3CH_2CH_2$), 2.19-2.40 (br s, 1H, $NHCH_2CO_2CH_2CH_3$), 3.06-3.18 (q, 2H, *J*=17.0 Hz, $NHCH_2CO_2CH_2CH_3$), 4.05-4.12 (q, 2H, *J*=7.1 Hz, $CO_2CH_2CH_3$), 6.03 (s, 1H, CONHH), 6.93 (s, 1H, CONHH), 7.15-7.30 (complex m, 3H, aromatic H), 7.39-7.46 (complex m, 2H, aromatic H); ^{13}C NMR (50 MHz, $CDCl_3$) *δ* 14.2 ($CH_3(CH_2)_2$), 14.4 ($CO_2CH_2CH_3$), 16.7 ($CH_3CH_2CH_2$), 35.8 ($CH_3CH_2CH_2$), 45.1 ($NHCH_2CO_2CH_2CH_3$), 61.1 ($CO_2CH_2CH_3$), 67.4 ($C_6H_5(n\text{-}C_3H_7)CNH$), 126.5, 127.5, 128.6 (2,3,4,5,6-aromatic C), 141.0 (1-aromatic C), 172.1 ($CO_2CH_2CH_3$), 176.7 ($CONH_2$); HRMS (ESI): $[M+H]^+$ calcd for $C_{15}H_{22}N_2O_3$, 279.1709, found 279.1705.

4.1.4. N-[(1-Aminocarbonyl-1-phenyl)pentyl]glycine ethyl ester **25**

Prepared from valerophenone **20** (4.32 g, 26.6 mmol) according to the method described for compound **23**. The crude oil was purified by flash column chromatography eluting with AcOEt-*n*-hexane 1:2 to 3:1 to afford the title compound **25** as a colorless viscous oil, which solidified on cooling (white crystals, 1.95 g, 25% overall yield): mp 72-74 °C (Et_2O-*n*-pentane); ^{1}H NMR (400 MHz, $CDCl_3$) *δ* 0.84 (t, 3H, *J*=7.3 Hz, $CH_3(CH_2)_3$), 1.05-1.17 (complex m,

2H, $CH_3CH_2CH_2CH_2$), 1.19 (t, 3H, *J*=7.1 Hz, $CO_2CH_2CH_3$), 1.26-1.39 (complex m, 2H, $CH_3CH_2(CH_2)_2$), 1.98-2.23 (dm, 2H, $CH_3(CH_2)_2CH_2$), 2.26-2.48 (br s, 1H, $NHCH_2CO_2CH_2CH_3$), 3.15 (~s, 2H, $NHCH_2CO_2CH_2CH_3$), 4.03-4.18 (q, 2H, *J*=7.1 Hz, $CO_2CH_2CH_3$), 6.64 (s, 1H, CONHH), 6.96 (s, 1H, CONHH), 7.16-7.32 (complex m, 3H, aromatic H), 7.41-7.50 (complex m, 2H, aromatic H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 13.9 ($CH_3(CH_2)_3$), 14.0 ($CO_2CH_2CH_3$), 22.8 ($CH_3CH_2(CH_2)_2$), 25.3 ($CH_3CH_2CH_2CH_2$), 33.1 ($CH_3(CH_2)_2CH_2$), 44.9 ($NHCH_2CO_2CH_2CH_3$), 60.9 ($CO_2CH_2CH_3$), 67.1 (C_6H_5(*n*-C_4H_9)CNH), 126.3, 127.3, 128.4 (2,3,4,5,6-aromatic C), 141.0 (1-aromatic C), 171.9 ($CO_2CH_2CH_3$), 176.8 ($CONH_2$). Anal. Calcd for $C_{16}H_{24}N_2O_3$: C, 65.72; H, 8.27; N, 9.58; Found: C, 65.95; H, 8.35; N, 9.39.

4.1.5. N-[[1-Aminocarbonyl-1-(4-fluorophenyl)]ethyl]glycine ethyl ester **26**

Prepared from 4-fluoroacetophenone **21** (3.67 g, 26.6 mmol) according to the method described for compound **23**. The crude oil was purified by column chromatography on silica gel eluting first with AcOEt-*n*-hexane 1:1 and then AcOEt to afford the title compound **26** as a light-yellow viscous oil, which solidified on standing (2.14 g, 30% overall yield). An analytical sample was obtained as white crystals upon dissolution of the product in Et_2O and *n*-pentane-mediated precipitation. Mp 90-92 °C. ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.23 (t, 3H, *J*=7.1 Hz, $CO_2CH_2CH_3$), 1.68 (s, 3H, CH_3), 2.24 (s, 1H, $NHCH_2CO_2CH_2CH_3$), 3.24 (~s, 2H, $NHCH_2CO_2CH_2CH_3$), 4.10-4.17 (q, 2H, *J*=7.1 Hz, $CO_2CH_2CH_3$), 6.18 (s, 1H, CONHH), 7.01 (t, 2H, *J*=8.7 Hz, 3,5-aromatic H), 7.03 (s, 1H, CONHH), 7.43-7.49 (~q, 2H, *J*=5.3 Hz, 2,6-aromatic H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 14.2 ($CO_2CH_2CH_3$), 23.1 (CH_3), 45.6 ($NHCH_2CO_2CH_2CH_3$), 61.3 ($CO_2CH_2CH_3$), 64.4 (4-$FC_6H_4(CH_3)$CNH), 115.2, 115.7 (d, $J_{C\text{-}F}$=21.2 Hz, 3,5-aromatic C), 128.2, 128.4 (d, $J_{C\text{-}F}$=8.1 Hz, 2,6-aromatic C), 137.6, 137.7 (d, $J_{C\text{-}F}$=3.3 Hz, 1-aromatic C), 159.8, 164.7 (d, $J_{C\text{-}F}$=245 Hz, 4-aromatic C), 172.3 ($CO_2CH_2CH_3$), 177.4 ($CONH_2$). Anal. Calcd for $C_{13}H_{17}FN_2O_3$: C, 58.20; H, 6.39; N, 10.44; Found: C, 58.05; H, 6.45; N, 10.62.

4.1.6. N-[[1-Aminocarbonyl-1-(4-nitrophenyl)]ethyl]glycine ethyl ester **27**

Prepared from 4-nitroacetophenone **22** (4.39 g, 26.6 mmol) according to the method described for compound **23**. The resulting crude oil was purified by column chromatography eluting first with AcOEt-*n*-hexane 1:1 to remove the starting 4-nitroacetophenone **22**. Further elution with AcOEt-*n*-hexane 2:1 followed by AcOEt afforded first the respective 2,6-diketopiperazine **46** (white crystals, 740 mg, 11% yield from **22**), and then the title compound **27** as an off-yellow viscous oil, which solidified on standing (4.33 g, 55% overall yield). An analytical sample was obtained as slightly off-yellow crystals upon dissolution of the product in AcOEt-Et_2O 1:5 and *n*-pentane-mediated precipitation. Mp 83-85 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.22 (t, 3H, *J*=7.2 Hz, $CO_2CH_2CH_3$), 1.72 (s, 3H, CH_3), 2.20-2.46 (br s, 1H, $NHCH_2CO_2CH_2CH_3$), 3.25 (~s, 2H, $NHCH_2CO_2CH_2CH_3$), 4.08-4.20 (q, 2H, *J*=7.1 Hz, $CO_2CH_2CH_3$), 6.34 (s, 1H, CONHH), 7.15 (s, 1H, CONHH), 7.68 (d, 2H, *J*=8.9 Hz, 2,6-aromatic H), 8.17 (d, 2H, *J*=8.9 Hz, 3,5-aromatic H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 14.2 ($CO_2CH_2CH_3$), 23.3 (CH_3), 45.6 ($NHCH_2CO_2CH_2CH_3$), 61.5 ($CO_2CH_2CH_3$), 65.0 (4-$NO_2C_6H_4(CH_3)$CNH), 123.7 (3,5-aromatic C), 127.7 (2,6-aromatic C), 147.3, 149.2 (1,4-aromatic C), 172.0 ($CO_2CH_2CH_3$), 176.2 ($CONH_2$). Anal. Calcd for $C_{13}H_{17}N_3O_5$: C, 52.87; H, 5.80; N, 14.23; Found: C, 52.59; H, 5.82; N, 14.52.

4.1.7. 3-Methyl-2,6-dioxo-3-phenyl-1-piperazineacetic acid benzyl ester **28**

A stirred solution of the amide-ester derivative **23** (1 g, 4 mmol) in dry THF (40 mL) was cooled in an ice-water bath and treated portionwise with potassium bis(trimethylsilyl)amide (798 mg, 4.0 mmol) under argon. After 2 h of stirring at room temperature under argon, the mixture was concentrated to dryness under reduced pressure, and the residue (potassium imidate salt) was dissolved in dry DMF (40 mL). To this solution, benzyl bromoacetate (962 mg, 4.2 mmol) dissolved in dry DMF (10 mL) was added dropwise, and the mixture was stirred at room temperature for 48 h under argon. The reaction mixture was then poured into ice-water (80 mL) and extracted with Et_2O (4 × 60 mL). The combined organic extracts were washed with brine (2 × 50 mL), dried (Na_2SO_4) and concentrated to dryness under reduced pressure. The remaining crude thick oil was purified by flash column chromatography with AcOEt-Et_2O-*n*-hexane 0.5:1:1, as eluent, to afford the title compound **28** as a pale pink viscous oil (1.13 g, 81%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.57 (s, 3H, CH_3), 2.10-2.39 (br s, 1H, 4-H), 3.40 (d, 1H, *J*=18.5 Hz, 5-H), 3.61 (d, 1H, *J*=18.5 Hz, 5-H), 4.45-4.65 (q, AB, 2H, J_{AB}=16.7 Hz, $CH_2CO_2CH_2Ph$), 5.11 (s, 2H,

CO_2CH_2Ph), 7.22-7.42 (m, 10H, aromatic H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.7 (CH_3), 40.3 ($CH_2CO_2CH_2Ph$), 46.0 (5-C), 62.6 (3-C), 67.3 (CO_2CH_2Ph), 125.7, 128.2, 128.4, 128.6, 128.9, 135.1, 138.7 (aromatic C), 167.7 (CO_2CH_2Ph), 171.3, 173.6 (2,6-C); HRMS ($APCI^+$): $[M+H]^+$ calcd for $C_{20}H_{20}N_2O_4$, 353.1501, found 353.1494.

4.1.8. 2,6-Dioxo-3-phenyl-3-propyl-1-piperazineacetic acid benzyl ester **29**

Prepared from the amide-ester derivative **24** (3.2 g, 11.5 mmol) following the procedure described for compound **28**. The crude yellow solid was purified by column chromatography on silica gel with AcOEt-*n*-hexane 1:2, as eluent, to afford the title compound **29** as a slightly off-yellow solid (3.98 g, 91%): mp 63-65 °C (Et_2O-*n*-pentane); ^1H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, 3H, *J*=7.2 Hz, $CH_3(CH_2)_2$), 1.20-1.38 (m, 2H, $CH_3CH_2CH_2$), 1.80-2.13 (dm, 2H, $CH_3CH_2CH_2$), 2.12-2.50 (br s, 1H, 4-H), 3.55 (d, 1H, *J*=18.4 Hz, 5-H), 3.74 (d, 1H, *J*=18.4 Hz, 5-H), 4.51-4.74 (q, AB, 2H, J_{AB}=16.7 Hz, $CH_2CO_2CH_2Ph$), 5.19 (s, 2H, CO_2CH_2Ph), 7.27-7.51 (complex m, 10H, aromatic H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 14.3 ($CH_3(CH_2)_2$), 17.3 ($CH_3CH_2CH_2$), 40.4 ($CH_2CO_2CH_2Ph$), 44.1 ($CH_3CH_2CH_2$), 46.0 (5-C), 65.9 (3-C), 67.4 (CO_2CH_2Ph), 126.2, 128.3, 128.4, 128.6, 128.8, 129.0, 135.3, 137.8 (aromatic C), 167.9 (CO_2CH_2Ph), 171.3, 173.2 (2,6-C). Anal. Calcd for $C_{22}H_{24}N_2O_4$: C, 69.45; H, 6.36; N, 7.36; Found: C, 69.67; H, 6.42; N, 7.07.

4.1.9. 3-Butyl-2,6-dioxo-3-phenyl-1-piperazineacetic acid benzyl ester **30**

Prepared from the amide-ester derivative **25** (2.5 g, 8.55 mmol) following the procedure described for compound **28**. The crude yellowish solid was purified by flash column chromatography with AcOEt-*n*-hexane 1:2, as eluent, to afford the title compound **30** as a white solid (2.81 g, 83%): mp 92-94 °C (Et_2O-*n*-pentane); ^1H NMR (400 MHz, $CDCl_3$) δ 0.78 (t, 3H, *J*=6.8 Hz, $CH_3(CH_2)_3$), 1.10-1.28 (m, 4H, $CH_3CH_2CH_2CH_2$), 1.76-2.08 (dm, 2H, $CH_3(CH_2)_2CH_2$), 2.09-2.42 (br s, 1H, 4-H), 3.47 (d, 1H, *J*=18.4 Hz, 5-H), 3.66 (d, 1H, *J*=18.4 Hz, 5-H), 4.47-4.62 (q, AB, 2H, J_{AB}=16.7 Hz, $CH_2CO_2CH_2Ph$), 5.11 (s, 2H, CO_2CH_2Ph), 7.15-7.45 (m, 10H, aromatic H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 14.0 ($CH_3(CH_2)_3$), 22.9 ($CH_3CH_2(CH_2)_2$), 26.0 ($CH_3CH_2CH_2CH_2$), 40.3 ($CH_2CO_2CH_2Ph$), 41.7 ($CH_3(CH_2)_2CH_2$), 45.9 (5-C), 65.8 (3-C), 67.4 (CO_2CH_2Ph), 126.2, 128.3, 128.6, 128.7, 128.9, 135.3, 137.8 (aromatic C), 167.8 (CO_2CH_2Ph), 171.3, 173.1 (2,6-C). Anal. Calcd for $C_{23}H_{26}N_2O_4$: C, 70.03; H, 6.64; N, 7.10; Found: C, 70.27; H, 6.80; N, 6.82.

4.1.10. 3-(4-Fluorophenyl)-3-methyl-2,6-dioxo-1-piperazineacetic acid benzyl ester **31**

Prepared from the amide-ester derivative **26** (2.5 g, 9.32 mmol) following the procedure described for compound **28**. The crude yellow oil was purified by flash column chromatography with AcOEt-*n*-hexane 1:2, as eluent, to afford the title compound **31** as a light-yellow viscous oil (2.52 g, 73%): ^1H NMR (400 MHz, $CDCl_3$) δ 1.63 (s, 3H, CH_3), 2.86 (br s, 1H, 4-H), 3.45 (d, 1H, *J*=18.6 Hz, 5-H), 3.70 (d, 1H, *J*=18.6 Hz, 5-H), 4.54-4.71 (q, AB, 2H, J_{AB}=16.7 Hz, $CH_2CO_2CH_2Ph$), 5.19 (s, 2H, CO_2CH_2Ph), 7.01 (t, 2H, *J*=8.7 Hz, 3,5-H for 4-FC_6H_4), 7.30-7.50 (complex m, 7H, C_6H_5, 2,6-H for 4-FC_6H_4); ^{13}C NMR (50 MHz, $CDCl_3$) δ 29.0 (CH_3), 40.4 ($CH_2CO_2CH_2Ph$), 45.9 (5-C), 62.3 (3-C), 67.5 (CO_2CH_2Ph), 115.7, 116.2 (d, J_{C-F}=21.4 Hz, 3,5-C for 4-FC_6H_4), 127.7, 127.9 (d, J_{C-F}=8.1 Hz, 2,6-C for 4-FC_6H_4), 128.4, 128.6, 128.8 (2,3,4,5,6-C for C_6H_5), 134.5, 134.6 (d, J_{C-F}=3.2 Hz, 1-C for 4-FC_6H_4), 135.2 (1-C for C_6H_5), 160.1, 165.0 (d, J_{C-F}=246 Hz, 4-C for 4-FC_6H_4), 167.8 (CO_2CH_2Ph), 171.2, 173.6 (2,6-C); HRMS (ESI): $[M+H]^+$, $[M+Na]^+$ calcd for $C_{20}H_{19}FN_2O_4$, 371.1407, 393.1227, found 371.1411, 393.1229.

4.1.11. 3-Methyl-3-(4-nitrophenyl)-2,6-dioxo-1-piperazineacetic acid 4-methoxybenzyl ester **32**

Prepared from the amide-ester derivative **27** (2 g, 6.77 mmol) following the procedure described for compound **28**, but with 4-methoxybenzyl bromoacetate instead of benzyl bromoacetate. The crude yellow solid was purified by column chromatography on silica gel eluting with AcOEt-*n*-hexane 1:1→1:0 to afford the title compound **32** as an off-yellow solid (2.4 g, 83%): mp 128-130 °C (AcOEt/Et_2O-*n*-pentane); ^1H NMR (400 MHz, $CDCl_3$) δ 1.67 (s, 3H, CH_3), 2.13-2.38 (br s, 1H, 4-H), 3.40 (d, 1H, *J*=18.7 Hz, 5-H), 3.76 (d, 1H, *J*=18.7 Hz, 5-H), 3.81 (s, 3H, OCH_3), 4.52-4.68 (q, AB, 2H, J_{AB}=16.7 Hz, $CH_2CO_2CH_2C_6H_4OCH_3$-4), 5.11 (s, 2H, $CO_2CH_2C_6H_4OCH_3$-4), 6.88 (d, 2H, *J*=8.7 Hz, 3,5-H for 4-$CH_3OC_6H_4$), 7.26 (d, 2H, *J*=8.7 Hz, 2,6-H for 4-$CH_3OC_6H_4$), 7.69 (d, 2H, *J*=9.0 Hz, 2,6-H for 4-$NO_2C_6H_4$), 8.16 (d, 2H, *J*=9.0 Hz, 3,5-H for 4-$NO_2C_6H_4$); ^{13}C NMR (50 MHz, $CDCl_3$) δ 29.0 (CH_3), 40.6 ($CH_2CO_2CH_2C_6H_4OCH_3$-4), 45.9 (5-C), 55.4 (OCH_3), 62.7 (3-C), 67.6 ($CO_2CH_2C_6H_4OCH_3$-4), 114.2, 124.3, 127.2,

130.3, 146.2, 148.0, 160.1 (aromatic C), 167.6 ($CO_2CH_2C_6H_4OCH_3$-4), 170.6, 172.7 (2,6-C). Anal. Calcd for $C_{21}H_{21}N_3O_7$: C, 59.01; H, 4.95; N, 9.83; Found: C, 58.78; H, 4.89; N, 10.02.

4.1.12. 3-Methyl-2,6-dioxo-3-phenyl-1-piperazineacetic acid **33**

10% Pd on charcoal (132 mg) was added to a solution of benzyl ester **28** (1.1 g, 3.12 mmol) in abs EtOH (78 mL). After 3 h of shaking under an atmosphere of 50 psi hydrogen at room temperature, the catalyst was removed by filtration and washed with EtOH (3 × 15 mL). The combined filtrates were concentrated to dryness under reduced pressure to afford the title compound **33** as a white foamy solid, which strongly binds the aforementioned solvent. Removal of the entrapped solvent upon drying at 62-64 °C under high vacuum (10^{-2} mmHg) in an Abderhalden apparatus gave **33** as a glass solid (814 mg, almost quantitative yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.67 (s, 3H, C*H*$_3$), 3.48 (d, 1H, *J*=18.5 Hz, 5-H), 3.75 (d, 1H, *J*=18.5 Hz, 5-H), 4.52-4.70 (q, AB, 2H, $J_{AB}\simeq$17.0 Hz, C*H*$_2$CO$_2$H), 7.0 (s, 2H, 4-H, CO$_2$*H*), 7.30-7.48 (m, 5H, aromatic H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 28.4 (*C*H$_3$), 40.2 (*C*H$_2$CO$_2$H), 45.8 (5-C), 62.8 (3-C), 125.7, 128.3, 129.0 (2,3,4,5,6-aromatic C), 138.4 (1-aromatic C) 171.1, 172.6, 173.4 (*C*O$_2$H, 2,6-C). The hydrochloride salt (**33·HCl**) was prepared by treating a clear diethyl ether solution of **33** with saturated solution of HCl in Et_2O under ice cooling. The white precipitate was collected by vacuum filtration, triturated with Et_2O and dried in vacuo. Mp 224-226 °C (dec). Anal. Calcd for $C_{13}H_{15}ClN_2O_4$: C, 52.27; H, 5.06; N, 9.38; Found: C, 51.85; H, 5.26; N, 9.62.

4.1.13. 2,6-Dioxo-3-phenyl-3-propyl-1-piperazineacetic acid **34**

Prepared from benzyl ester **29** (2.46 g, 6.47 mmol) by catalytic hydrogenolysis (H_2/10% Pd-C, 295 mg) in abs EtOH (160 mL) as described for compound **33**. The resulting off-white foamy solid was triturated with a diethyl ether-*n*-pentane 1:1 mixture giving the title compound **34** as white crystals (1.84 g, 98%). Recrystallization from Et_2O-*n*-pentane furnished an analytical sample, which had mp 131-133 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, 3H, $J\simeq$7.2 Hz, C*H*$_3$(CH$_2$)$_2$), 1.22-1.38 (m, 2H, CH$_3$C*H*$_2$CH$_2$), 1.83-2.14 (dm, 2H, CH$_3$CH$_2$C*H*$_2$), 3.55 (d, 1H, *J*=18.4 Hz, 5-H), 3.77 (d, 1H, *J*=18.4 Hz, 5-H), 4.50-4.70 (q, AB, 2H, J_{AB}=17.0 Hz, C*H*$_2$CO$_2$H), 6.65 (s, 2H, 4-H, CO$_2$*H*), 7.22-7.48 (m, 5H, aromatic H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 14.3 (*C*H$_3$(CH$_2$)$_2$), 17.3 (CH$_3$*C*H$_2$CH$_2$), 40.1 (*C*H$_2$CO$_2$H), 43.9 (CH$_3$CH$_2$*C*H$_2$), 45.8 (5-C), 65.9 (3-C), 126.2, 128.4, 129.0 (2,3,4,5,6-aromatic C), 137.5 (1-aromatic C), 171.3, 173.0 (*C*O$_2$H, 2,6-C). Anal. Calcd for $C_{15}H_{18}N_2O_4$: C, 62.06; H, 6.25; N, 9.65; Found: C, 61.79; H, 6.30; N, 9.96.

4.1.14. 3-Butyl-2,6-dioxo-3-phenyl-1-piperazineacetic acid **35**

Prepared from benzyl ester **30** (2.7 g, 6.84 mmol) by catalytic hydrogenolysis (H_2/10% Pd-C, 324 mg) in abs EtOH-AcOEt 3:1 (170 mL) as described for compound **33**. The resulting glass solid was triturated with Et_2O (50 mL) to give the title compound **35** as an off-white crystalline solid (2 g, 96%). Recrystallization of specimen of this material from Et_2O-*n*-pentane furnished an analytical sample as white crystals, mp 121-123 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.86 (t, 3H, *J*=6.6 Hz, C*H*$_3$(CH$_2$)$_3$), 1.18-1.37 (m, 4H, CH$_3$C*H*$_2$C*H*$_2$CH$_2$), 1.84-2.18 (sym dm, 2H, CH$_3$(CH$_2$)$_2$C*H*$_2$), 3.56 (d, 1H, *J*=18.5 Hz, 5-H), 3.77 (d, 1H, *J*=18.5 Hz, 5-H), 4.52-4.70 (q, AB, 2H, J_{AB}=17.0 Hz, C*H*$_2$CO$_2$H), 6.80 (s, 2H, 4-H, CO$_2$*H*), 7.25-7.52 (m, 5H, aromatic H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 14.0 (*C*H$_3$(CH$_2$)$_3$), 23.0 (CH$_3$*C*H$_2$(CH$_2$)$_2$), 26.1 (CH$_3$CH$_2$*C*H$_2$CH$_2$), 40.1 (*C*H$_2$CO$_2$H), 41.6 (CH$_3$(CH$_2$)$_2$*C*H$_2$), 45.8 (5-C), 65.9 (3-C), 126.2, 128.3, 129.0 (2,3,4,5,6-aromatic C), 137.6 (1-aromatic C), 171.3, 173.0, 173.4 (*C*O$_2$H, 2,6-C). Anal. Calcd for $C_{16}H_{20}N_2O_4$: C, 63.14; H, 6.62; N, 9.20; Found: C, 62.90; H, 6.68; N, 9.48.

4.1.15. 3-(4-Fluorophenyl)-3-methyl-2,6-dioxo-1-piperazineacetic acid **36**

Prepared from benzyl ester **31** (2.47 g, 6.67 mmol) by catalytic hydrogenolysis (H_2/10% Pd-C, 296 mg) in abs EtOH (167 mL) as described for compound **33**. The obtained white foamy solid strongly binds the hydrogenation solvent. Removal of the entrapped solvent as in **33** gave the title compound **36** as a white amorphous solid (1.86 g, almost quantitative yield). A small quantity of this material was dissolved in AcOEt-Et_2O 1:1, and the clear solution was concentrated to dryness in vacuo. Trituration of the residue with a diethyl ether-*n*-pentane 1:1 mixture provided an analytical sample as white crystals. Mp 150-153 °C. ^{1}H NMR (400 MHz, DMS0-d_6) δ 1.50 (s, 3H, C*H*$_3$), 3.16 (d, 1H, *J*=18.4 Hz, 5-H), 3.62 (d, 1H, *J*=18.4 Hz, 5-H), 3.25-4.25 (v br s, 2H, 4-H, CO$_2$*H*), 4.30-4.45 (q, AB, 2H, $J_{AB}\simeq$17.3 Hz, C*H*$_2$CO$_2$H), 7.19 (t like, 2H, *J*=7.3, 8.4 Hz, 3,5-aromatic H), 7.47 (t like, 2H, *J*=5.3, 7.5 Hz, 2,6-

aromatic H); ^{13}C NMR (50 MHz, DMSO-d_6) δ 28.3 (*C*H_3), 40.2 (*C*H_2CO_2H), 45.6 (5-C), 61.6 (3-C), 115.2, 115.7 (d, $J_{C\text{-}F}$=21.2 Hz, 3,5-aromatic C), 128.0, 128.2 (d, $J_{C\text{-}F}$=7.9 Hz, 2,6-aromatic C), 136.2 (1-aromatic C), 159.2, 164.1 (d, $J_{C\text{-}F}$=243 Hz, 4-aromatic C), 169.1 (*C*O_2H), 171.3, 173.5 (2,6-C). Anal. Calcd for $C_{13}H_{13}FN_2O_4$: C, 55.71; H, 4.68; N, 10.0; Found: C, 55.98; H, 4.46; N, 9.66.

4.1.16. 3-Methyl-3-phenyl-2,6-piperazinedione **42**

The amide-ester derivative **23** (500 mg, 2 mmol) was subjected to treatment with potassium bis(trimethylsilyl)amide (399 mg, 2.0 mmol) in dry THF (20 mL) as described in **28**. The reaction was then quenched by adding trifluoroacetic acid (230 mg, 2 mmol, 1 equiv), and the solvent was evaporated to dryness under reduced pressure. Purification of the residual viscous oil by column chromatography on silica gel with AcOEt-Et_2O-*n*-hexane 1:1:0.5, as eluent, afforded the title compound **42** as a white crystalline solid (380 mg, 93%): mp 131-133 °C (AcOEt/Et_2O-*n*-pentane) (lit[10] mp 133-133.5 °C); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.63 (s, 3H, C*H*$_3$), 2.31 (s, 1H, 4-H), 3.38 (d, 1H, *J*=18.6 Hz, 5-H), 3.62 (d, 1H, *J*=18.6 Hz, 5-H), 7.27-7.53 (complex m, 5H, aromatic H), 8.79 (s, 1H, 1-H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 28.1 (*C*H_3), 45.9 (5-C), 62.3 (3-C), 125.7, 128.4, 129.2 (2,3,4,5,6-aromatic C), 138.6 (1-aromatic C) 172.9, 174.5 (2,6-C). Anal. Calcd for $C_{11}H_{12}N_2O_2$: C, 64.69; H, 5.92; N, 13.72; Found: C, 64.66; H, 5.82; N, 13.88.

4.1.17. 3-Phenyl-3-propyl-2,6-piperazinedione **43**

Prepared from the amide-ester derivative **24** (1.17 g, 4.2 mmol) in the same manner as for **42**. The resulting crude oil was purified by column chromatography on silica gel with AcOEt-*n*-hexane 1:1, as eluent, to afford the title compound **43** as a glass oil (930 mg, 95%). Crystallization of this product material upon treatment with a diethyl ether-*n*-pentane 1:1 mixture, and subsequent recrystallization (Et_2O-*n*-pentane) gave a white crystalline solid, which melted at 80-82 °C (lit[10] mp 75-77.5 °C) after drying at room temperature in vacuo. This solid product strongly binds Et_2O as indicated by its ^{1}H NMR spectrum. Removal of the crystal solvent upon drying at 62-64 °C under high vacuum (10^{-2} mmHg) in an Abderhalden apparatus restored the solid material to the initial glass oil state. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.8 (t, 3H, *J*=7.3 Hz, C*H*$_3$(CH_2)$_2$), 1.10-1.32 (m, 2H, $CH_3CH_2CH_2$), 1.77-2.0 (dm, 2H, $CH_3CH_2CH_2$), 2.09-2.32 (br s, 1H, 4-H), 3.39 (d, 1H, *J*=18.5 Hz, 5-H), 3.58 (d, 1H, *J*=18.5 Hz, 5-H), 7.17-7.47 (m, 5H, aromatic H), 8.53 (s, 1H, 1-H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 14.3 (*C*H_3(CH_2)$_2$), 17.3 ($CH_3CH_2CH_2$), 43.2 ($CH_3CH_2CH_2$), 45.7 (5-C), 65.3 (3-C), 126.1, 128.3, 129.0 (2,3,4,5,6-aromatic C), 137.4 (1-aromatic C), 172.7, 174.0 (2,6-C); ES$^+$ MS:m/z 233.2 [M+H]$^+$.

4.1.18. 3-Butyl-3-phenyl-2,6-piperazinedione **44**

Prepared from the amide-ester derivative **25** (2.24 g, 7.66 mmol) in the same manner as for **42**. The resulting crude oil was purified by flash column chromatography eluting with AcOEt-*n*-hexane 1:2 to afford the title compound **44** as a slightly off-white solid (1.81 g, 96%). To obtain an analytical sample, a small quantity of this material was recrystallized from Et_2O-*n*-pentane giving a cotton like solid, which melted at 72-74 °C after drying at room temperature in vacuo. This solid material strongly binds the recrystallization solvents as indicated by its ^{1}H NMR spectrum. Removal of the crystal solvents upon drying at 62-64 °C under high vacuum (10^{-2} mmHg) provided a white crystalline solid, which had mp 79-82 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.86 (t, 3H, *J*=6.8 Hz, C*H*$_3$(CH_2)$_3$), 1.16-1.37 (m, 4H, $CH_3CH_2CH_2CH_2$), 1.83-2.13 (dm, 2H, $CH_3(CH_2)_2CH_2$), 2.18-2.36 (br s, 1H, 4-H), 3.47 (d, 1H, *J*=18.1 Hz, 5-H), 3.66 (d, 1H, *J*=18.5 Hz, 5-H), 7.28-7.53 (m, 5H, aromatic H), 7.96 (s, 1H, 1-H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 14.0 (*C*H_3(CH_2)$_3$), 22.9 ($CH_3CH_2(CH_2)_2$), 26.1 ($CH_3CH_2CH_2CH_2$), 40.9 ($CH_3(CH_2)_2CH_2$), 45.8 (5-C), 65.3 (3-C), 126.1, 128.3, 129.1 (2,3,4,5,6-aromatic C), 137.5 (1-aromatic C), 172.4, 173.8 (2,6-C). Anal. Calcd for $C_{14}H_{18}N_2O_2$: C, 68.27; H, 7.37; N, 11.37; Found: C, 68.42; H, 7.64; N, 11.09.

4.1.19. 3-(4-Fluorophenyl)-3-methyl-2,6-piperazinedione **45**

Prepared from the amide-ester derivative **26** (500 mg, 1.86 mmol) in the same manner as for **42**. The resulting crude solid was purified by flash column chromatography eluting with AcOEt-*n*-hexane 2:1 to afford the title compound **45** as a white crystalline solid (380 mg, 92%): mp 151-153 °C. (AcOEt/Et_2O 1:20-*n*-pentane); ^{1}H NMR (600 MHz, DMSO-d_6) δ 1.43 (s, 3H, C*H*$_3$), 2.98-3.04 (q, 1H, *J*=10.7 Hz, 5-H), 3.40-3.45 (dd, 1H, *J*=4.5, 18.3 Hz, 5-

H), 3.79-3.83 (dd, 1H, *J*=4.5, 10.6 Hz, 4-H), 7.21 (~t, 2H, *J*=8.8 Hz, 3,5-aromatic H), 7.40-7.47 (~q, 2H, *J*=5.5 Hz, 2,6-aromatic H), 11.07 (s, 1H, 1-H); ^{13}C NMR (50 MHz, DMSO-d_6) δ 27.7 (*C*H_3), 45.4 (5-C), 60.8 (3-C), 115.4, 115.8 (d, $J_{C\text{-}F}$=21.3 Hz, 3,5-aromatic C), 127.5, 127.7 (d, $J_{C\text{-}F}$=8.1 Hz, 2,6-aromatic C), 136.3 (1-aromatic C), 159.1, 164.0 (d, $J_{C\text{-}F}$=242 Hz, 4-aromatic C), 173.1, 174.8 (2,6-C). Anal. Calcd for $C_{11}H_{11}FN_2O_2$: C, 59.45; H, 4.99; N, 12.61; Found: C, 59.41; H, 4.89; N, 12.76.

4.1.20. 3-Methyl-3-(4-nitrophenyl)-2,6-piperazinedione **46**

Prepared from the amide-ester derivative **27** (1 g, 3.39 mmol) in the same manner as for **42**. The resulting crude solid was purified by column chromatography on silica gel with AcOEt-*n*-hexane 2:1, as eluent, to afford the title compound **46** as an off-yellow solid (770 mg, 91%): mp 211-213 °C. (MeOH-Et_2O); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.47 (s, 3H, C*H*$_3$), 2.98-3.11 (q, 1H, *J*=10.8 Hz, 5-H), 3.42-3.52 (dd, 1H, *J*=4.5, 18.4 Hz, 5-H), 3.92-4.02 (dd, 1H, *J*=4.4, 10.7 Hz, 4-H), 7.69 (d, 2H, *J*=8.8 Hz, 2,6-aromatic H), 8.25 (d, 2H, *J*=8.0 Hz, 3,5-aromatic H), 11.22 (s, 1H, 1-H); ^{13}C NMR (50 MHz, DMSO-d_6) δ 27.4 (*C*H_3), 45.4 (5-C), 61.3 (3-C), 124.0 (3,5-aromatic C), 127.0 (2,6-aromatic C), 147.1, 148.0 (1,4-aromatic C), 172.8, 174.1 (2,6-C). Anal. Calcd for $C_{11}H_{11}N_3O_4$: C, 53.01; H, 4.45; N, 16.86; Found: C, 52.88; H, 4.61; N, 17.04.

4.1.21. 3,4-Dimethyl-3-phenyl-2,6-piperazinedione **47**

A solution of compound **42** (721 mg, 3.53 mmol) and aqueous formaldehyde 37% (2.2 mL) in methanol (22 mL) was stirred at room temperature for 3 h, and $NaCNBH_3$ (398 mg, 6.33 mmol) was then added in one portion. After 20 min of stirring, the pH of the reaction mixture was adjusted to 6-7 by dropwise addition of acetic acid. Stirring was continued for 4 h at room temperature with occasional addition of acetic acid to maintain the pH at 6-7. Methanol was removed by evaporation in vacuo, and the residue was treated with water (10 mL) followed by 1N aq NaOH and solid Na_2CO_3 until the pH was adjusted to 8. Subsequently the mixture was extracted with CH_2Cl_2 (3 ×35 mL), and the combined organic extracts were washed with brine (2 ×35 mL), dried (Na_2SO_4) and concentrated to dryness under reduced pressure. The remaining viscous oil solidified after a few minutes of standing. The resulting crude solid was purified over a column of flash silica eluting with AcOEt-*n*-hexane-Et_2O 1:1:0.5 to afford the title compound **47** as a white crystalline solid (593 mg, 77%): mp 127-129 °C. (Et_2O-*n*-pentane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.63 (s, 3H, 3-C*H*$_3$), 2.52 (s, 3H, 4-C*H*$_3$), 3.27-3.61 (q, AB, 2H, J_{AB}=18.2 Hz, 5-H), 7.27-7.47 (m, 5H, aromatic H), 8.79 (br s, 1H, 1-H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 23.5 (3-*C*H_3), 38.3 (4-*C*H_3), 54.9 (5-C), 67.7 (3-C), 126.0, 128.3, 129.0 (2,3,4,5,6-aromatic C), 140.5 (1-aromatic C), 171.4, 174.1 (2,6-C). Anal. Calcd for $C_{12}H_{14}N_2O_2$: C, 66.04; H, 6.47; N, 12.84; Found: C, 65.91; H, 6.62; N, 12.62.

4.1.22. 4-Methyl-3-phenyl-3-propyl-2,6-piperazinedione **48**

Prepared by reductive methylation of diketopiperazine **43** (920 mg, 3.96 mmol) as described for compound **47**. The crude thick oil was purified by column chromatography on silica gel with AcOEt-*n*-hexane 1:3, as eluent, to afford a colorless viscous oil, which solidified on standing (white crystals, 860 mg, 88% yield): mp 116-118 °C (Et_2O-*n*-pentane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.75 (t, 3H, *J*=7.4 Hz, C*H*$_3$(CH$_2$)$_2$), 1.02-1.20 (m, 2H, CH$_3$C*H*$_2$CH$_2$), 1.77-2.08 (dm, 2H, CH$_3$CH$_2$C*H*$_2$), 2.43 (s, 3H, 4-C*H*$_3$), 3.21-3.66 (q, AB, 2H, J_{AB}=18.2 Hz, 5-H), 7.17-7.36 (m, 5H, aromatic H), 8.82 (s, 1H, 1-H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 14.3 (*C*H$_3$(CH$_2$)$_2$), 16.2 (CH$_3$*C*H$_2$CH$_2$), 38.1 (4-*C*H$_3$), 38.3 (CH$_3$CH$_2$*C*H$_2$), 55.1 (5-C), 70.0 (3-C), 126.9, 128.2, 128.7 (2,3,4,5,6-aromatic C), 136.9 (1-aromatic C), 171.4, 174.0 (2,6-C). Anal. Calcd for $C_{14}H_{18}N_2O_2$: C, 68.27; H, 7.37; N, 11.37; Found: C, 68.41; H, 7.21; N, 11.51.

4.1.23. 3-Butyl-4-methyl-3-phenyl-2,6-piperazinedione **49**

Prepared by reductive methylation of diketopiperazine **44** (900 mg, 3.65 mmol) as described for compound **47**. The crude yellowish thick oil was purified over a column of flash silica eluting with AcOEt-*n*-hexane 1:4 to afford the title compound **49** as a colorless viscous oil, which solidified on standing under cooling (white crystals, 810 mg, 85% yield): mp 67-69 °C. (Et_2O-*n*-pentane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.82 (t, 3H, *J*≃7.0 Hz, C*H*$_3$(CH$_2$)$_3$), 1.05-1.31 (m, 4H, CH$_3$C*H*$_2$C*H*$_2$CH$_2$), 1.89-2.15 (dm, 2H, CH$_3$(CH$_2$)$_2$C*H*$_2$), 2.50 (s, 3H, 4-C*H*$_3$), 3.26-3.76 (q, AB, 2H, J_{AB}=18.2 Hz, 5-H), 7.30-7.52 (m, 5H, aromatic H), 8.48 (s, 1H, 1-H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 14.0 (*C*H$_3$(CH$_2$)$_3$), 22.9 (CH$_3$*C*H$_2$(CH$_2$)$_2$), 24.8 (CH$_3$CH$_2$*C*H$_2$CH$_2$), 35.8 (CH$_3$(CH$_2$)$_2$*C*H$_2$), 38.1 (4-*C*H$_3$), 55.1 (5-C), 70.1 (3-C), 126.9, 128.3,

128.7 (2,3,4,5,6-aromatic C), 136.8 (1-aromatic C), 171.2, 173.9 (2,6-C). Anal. Calcd for $C_{15}H_{20}N_2O_2$: C, 69.20; H, 7.74; N, 10.76; Found: C, 69.32; H, 7.63; N, 10.98.

4.1.24. 3-(4-Fluorophenyl)-3,4-dimethyl-2,6-piperazinedione **50**

Prepared by reductive methylation of diketopiperazine **45** (1.05 g, 4.73 mmol) as described for compound **47**. The crude yellowish thick oil was purified by column chromatography on silica gel with AcOEt-*n*-hexane 1:3, as eluent, to afford the title compound as a colorless viscous oil, which solidified on standing (white crystals, 765 mg, 69% yield): mp 116-118 °C. (Et_2O-*n*-pentane); ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.60 (s, 3H, 3-CH_3), 2.48 (s, 3H, 4-CH_3), 3.30-3.57 (q, AB, 2H, J_{AB}=18.1 Hz, 5-H), 7.04 (t like, 2H, J=8.6 Hz, 3,5-aromatic H), 7.40-7.45 (q like, 2H, J=5.2 Hz, 2,6-aromatic H), 8.64 (s, 1H, 1-H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 23.0 (3-CH$_3$), 38.3 (4-CH$_3$), 54.7 (5-C), 67.1 (3-C), 115.7, 116.1 (d, $J_{C\text{-}F}$=21.4 Hz, 3,5-aromatic C), 127.8, 128.0 (d, $J_{C\text{-}F}$=7.9 Hz, 2,6-aromatic C), 136.3 (1-aromatic C), 160.0, 165.0 (d, $J_{C\text{-}F}$=246 Hz, 4-aromatic C), 171.1, 174.0 (2,6-C). Anal. Calcd for $C_{12}H_{13}FN_2O_2$: C, 61.01; H, 5.55; N, 11.86; Found: C, 61.18; H, 5.45; N, 12.08.

4.1.25. 3,4-Dimethyl-3-(4-nitrophenyl)-2,6-piperazinedione **51**

Prepared by reductive methylation of diketopiperazine **46** (1.06 g, 4.25 mmol) in THF-MeOH 1:1 (50 mL) as described for compound **47**. The crude viscous was purified by column chromatography on silica gel with AcOEt-*n*-hexane 1:4 to 1:2 to afford the title compound **51** as a white crystalline solid (925 mg, 83%): mp 136-138 °C. (AcOEt/Et_2O-*n*-pentane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.65 (s, 3H, 3-CH_3), 2.50 (s, 3H, 4-CH_3), 3.36-3.57 (q, AB, 2H, J_{AB}=18.1 Hz, 5-H), 7.69 (d, 2H, J=9.0 Hz, 2,6-aromatic H), 8.23 (d, 2H, J=9.0 Hz, 3,5-aromatic H), 8.55 (s, 1H, 1-H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 22.4 (3-CH$_3$), 38.3 (4-CH$_3$), 54.7 (5-C), 67.5 (3-C), 124.3 (3,5-aromatic C), 127.4 (2,6-aromatic C), 148.0 (1,4-aromatic C), 170.2, 173.0 (2,6-C). Anal. Calcd for $C_{12}H_{13}N_3O_4$: C, 54.75; H, 4.98; N, 15.96; Found: C, 54.59; H, 4.82; N, 16.14.

4.1.26. 3,4-Dimethyl-2,6-dioxo-3-phenyl-1-piperazineacetic acid benzyl ester **52**

A stirred solution of diketopiperazine **47** (1.13 g, 5.18 mmol) in dry DMF (30 mL) was treated portionwise with sodium hydride (149 mg, 6.22 mmol). After 1 h of stirring at room temperature under argon, benzyl bromoacetate (1.25 g, 5.43 mmol) dissolved in dry DMF (6 mL) was added dropwise. Stirring was continued at room temperature for 48 h under argon, and the reaction mixture was then poured into ice-water (75 mL) and extracted with ethyl acetate (4 × 60 mL). The combined organic extracts were washed with brine (2 × 60 mL), dried (Na_2SO_4), and concentrated to dryness in vacuo. The residual thick oil was purified by flash column chromatography eluting with AcOEt-*n*-hexane 1:2 to afford the title compound **52** as a light-yellow viscous oil, which solidified on standing under cooling (1.62 g, 85%). An analytical sample was obtained as white crystals upon dissolution of the product in Et_2O and *n*-pentane-mediated precipitation. Mp 90-92 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.65 (s, 3H, 3-CH_3), 2.54 (s, 3H, 4-CH_3), 3.37-3.72 (q, AB, 2H, J_{AB}=18.0 Hz, 5-H), 4.59-4.75 (q, AB, 2H, J_{AB}=16.8 Hz, CH_2CO$_2$CH$_2$Ph), 5.20 (s, 2H, CO$_2$CH_2Ph), 7.25-7.47 (complex m, 10H, aromatic H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 24.4 (3-CH$_3$), 38.1 (4-CH$_3$), 40.3 (CH$_2$CO$_2$CH$_2$Ph), 55.1 (5-C), 67.5 (CO$_2$$CH_2$Ph), 68.0 (3-C), 126.1, 128.3, 128.5, 128.6, 128.7, 129.0, 135.2, 140.7 (aromatic C), 167.8 (CO$_2$CH$_2$Ph), 170.4, 173.7 (2,6-C). Anal. Calcd for $C_{21}H_{22}N_2O_4$: C, 68.84; H, 6.05; N, 7.65; Found: C, 69.24; H, 6.43; N, 8.01.

4.1.27. 4-Methyl-2,6-dioxo-3-phenyl-3-propyl-1-piperazineacetic acid benzyl ester **53**

Prepared from diketopiperazine **48** (1.15 g, 4.67 mmol) following the procedure described for compound **52**. The crude oil was purified by column chromatography on silica gel with AcOEt-petroleum ether (44-60 °C) 1:6, as eluent, to afford the title compound **53** as a slightly off-yellow viscous oil (1.35 g, 75%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.75 (t, 3H, $J\simeq$7.3 Hz, CH_3(CH$_2$)$_2$), 1.04-1.15 (m, 2H, CH$_3$CH_2CH$_2$), 1.78-2.09 (dm, 2H, CH$_3$CH$_2$CH_2), 2.42 (s, 3H, 4-CH_3), 3.28-3.71 (q, AB, 2H, J_{AB}=18.1 Hz, 5-H), 4.47-4.63 (q, AB, 2H, J_{AB}=16.7 Hz, CH_2CO$_2$CH$_2$Ph), 5.06-5.16 (q, AB, 2H, J_{AB}=12.3 Hz, CO$_2$CH_2Ph), 7.16-7.34 (complex m, 10H, aromatic H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 14.3 (CH$_3$(CH$_2$)$_2$), 16.3 (CH$_3$$CH_2CH_2$), 37.8 (4-$CH_3$), 39.0 (CH$_3CH_2$$CH_2$), 40.4 ($CH_2CO_2CH_2$Ph), 55.1 (5-C), 67.4 (CO$_2$CH$_2$Ph), 70.3 (3-C), 127.0, 128.2, 128.4, 128.6, 128.7, 135.2, 136.9 (aromatic C), 167.8 (CO$_2$CH$_2$Ph), 170.4, 173.6 (2,6-C); HRMS (ESI): $[M+H]^+$ calcd for $C_{23}H_{26}N_2O_4$, 395.1971, found 395.1967.

4.1.28. 3-Butyl-4-methyl-2,6-dioxo-3-phenyl-1-piperazineacetic acid benzyl ester **54**

Prepared from diketopiperazine **49** (1.45 g, 5.57 mmol) following the procedure described for compound **52**. The crude oil was purified by flash column chromatography eluting with AcOEt-*n*-hexane 1:3 to afford the title compound **54** as a colorless viscous oil (1.97 g, 87%): ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.75 (t, 3H, J=7.2 Hz, CH_3(CH$_2$)$_3$), 1.04-1.20 (dm, 4H, CH$_3$CH_2CH_2CH$_2$), 1.85-2.09 (dm, 2H, CH$_3$(CH$_2$)$_2$CH_2), 2.42 (s, 3H, 4-CH_3), 3.30-3.69 (q, AB, 2H, J_{AB}=18.1 Hz, 5-H), 4.49-4.61 (q, AB, 2H, J_{AB}=16.7 Hz, CH_2CO$_2$CH$_2$Ph), 5.08-5.14 (q, AB, 2H, J_{AB}=12.2 Hz, CO$_2$CH_2Ph), 7.16-7.32 (complex m, 10H, aromatic H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 14.0 (*C*H$_3$(CH$_2$)$_3$), 22.9 (CH$_3$*C*H$_2$(CH$_2$)$_2$), 24.9 (CH$_3$CH$_2$*C*H$_2$CH$_2$), 36.5 (CH$_3$(CH$_2$)$_2$*C*H$_2$), 37.8 (4-*C*H$_3$), 40.4 (*C*H$_2$CO$_2$CH$_2$Ph), 55.1 (5-C), 67.4 (CO$_2$*C*H$_2$Ph), 70.3 (3-C), 127.0, 128.2, 128.4, 128.6, 128.7, 135.2, 136.8 (aromatic C), 167.8 (*C*O$_2$CH$_2$Ph), 170.4, 173.7 (2,6-C); HRMS (ESI): [M+H]$^+$ calcd for $C_{24}H_{28}N_2O_4$, 409.2127, found 409.2118.

4.1.29. 3-(4-Fluorophenyl)-3,4-dimethyl-2,6-dioxo-1-piperazineacetic acid benzyl ester **55**

Prepared from piperazine **50** (1.53 g, 6.48 mmol) following the procedure described for compound **52**. The crude oil was purified by column chromatography on silica gel with AcOEt-*n*-hexane 1:2, as eluent, to afford the title compound **55** as a colorless viscous oil (1.78 g, 71%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.62 (s, 3H, 3-CH_3), 2.50 (s, 3H, 4-CH_3), 3.37-3.68 (q, AB, 2H, J_{AB}=18.0 Hz, 5-H), 4.56-4.73 (q, AB, 2H, J_{AB}=16.8 Hz, CH_2CO$_2$CH$_2$Ph), 5.19 (s, 2H, CO$_2$CH_2Ph), 7.0 (t, 2H, J=8.7 Hz, 3,5-H for 4-FC$_6$H$_4$), 7.30-7.44 (complex m, 7H, C$_6H_5$, 2,6-H for 4-FC$_6$H$_4$); ^{13}C NMR (50 MHz, $CDCl_3$) δ 24.0 (3-*C*H$_3$), 38.1 (4-*C*H$_3$), 40.4 (*C*H$_2$CO$_2$CH$_2$Ph), 54.9 (5-C), 67.5 (CO$_2$*C*H$_2$Ph), 69.1 (3-C), 115.6, 116.0 (d, $J_{C\text{-}F}$=21.3 Hz, 3,5-C for 4-FC$_6$H$_4$), 128.0, 128.1 (d, $J_{C\text{-}F}$=7.9 Hz, 2,6-C for 4-FC$_6$H$_4$), 128.5, 128.7, 128.8 (2,3,4,5,6-C for C$_6$H$_5$), 135.2, 136.5 (1-C for 4-FC$_6$H$_4$ and C$_6$H$_5$), 160.0, 164.9 (d, $J_{C\text{-}F}$=246 Hz, 4-C for 4-FC$_6$H$_4$), 167.7 (*C*O$_2$CH$_2$Ph), 170.1, 173.5 (2,6-C); HRMS (ESI): [M+H]$^+$ calcd for $C_{21}H_{21}FN_2O_4$, 385.1564, found 385.1576.

4.1.30. 3,4-Dimethyl-3-(4-nitrophenyl)-2,6-dioxo-1-piperazineacetic acid 4-methoxybenzyl ester **56**

Sodium hydride (92 mg, 3.83 mmol) was added in portions to a stirred solution of diketopiperazine **51** (840 mg, 3.19 mmol) in dry DMF (20 mL), and the mixture was left stirring for 10 min at room temperature under argon. In this time window, the mixture color changed from light yellow to blue-red. Then, 4-methoxybenzyl bromoacetate (868 mg, 3.35 mmol) dissolved in dry DMF (5 mL) was added dropwise, and the mixture was stirred at room temperature for 48 h under argon. The reaction was then worked up in the same way described for the preparation of **52**. The resulting orange thick oil was purified by column chromatography on silica gel eluting with AcOEt-*n*-hexane 1:3 and then 1:2 to afford the title compound **56** as a clear, yellow-orange viscous oil (1.18 g, 84%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.65 (s, 3H, 3-CH_3), 2.51 (s, 3H, 4-CH_3), 3.40-3.64 (q, AB, 2H, J_{AB}=18.1 Hz, 5-H), 3.80 (s, 3H, OCH_3), 4.55-4.69 (q, AB, 2H, J_{AB}=16.7 Hz, CH_2CO$_2$CH$_2$C$_6$H$_4$OCH$_3$-4), 5.11 (s, 2H, CO$_2$CH_2C$_6$H$_4$OCH$_3$-4), 6.88 (d, 2H, J=8.7 Hz, 3,5-H for 4-CH$_3$OC$_6$H$_4$), 7.26 (d, 2H, J=8.7 Hz, 2,6-H for 4-CH$_3$OC$_6$H$_4$), 7.65 (d, 2H, J=9.0 Hz, 2,6-H for 4-NO$_2$C$_6$H$_4$), 8.15 (d, 2H, J=9.0 Hz, 3,5-H for 4-NO$_2$C$_6$H$_4$); ^{13}C NMR (50 MHz, $CDCl_3$) δ 23.5 (3-*C*H$_3$), 38.1 (4-*C*H$_3$), 40.4 (*C*H$_2$CO$_2$CH$_2$C$_6$H$_4$OCH$_3$-4), 54.7 (5-C), 55.3 (OCH$_3$), 67.5 (3-C, CO$_2$*C*H$_2$C$_6$H$_4$OCH$_3$-4), 114.1, 124.1, 127.5, 130.3, 147.8, 148.0, 160.0 (aromatic C), 167.6 (*C*O$_2$CH$_2$C$_6$H$_4$OCH$_3$-4), 169.4, 172.5 (2,6-C); HRMS (ESI): [M+Na]$^+$ calcd for $C_{22}H_{23}N_3O_7$, 464.1434, found 464.1424.

4.1.31. 3,4-Dimethyl-2,6-dioxo-3-phenyl-1-piperazineacetic acid **57**

Prepared from benzyl ester **52** (1.3 g, 3.55 mmol) by catalytic hydrogenolysis (H_2/10% Pd-C, 156 mg) in abs EtOH (90 mL) as described for compound **33**. The obtained white foamy solid strongly binds the hydrogenation solvent. Removal of the entrapped solvent as in **33** gave the title compound **57** as a glass solid (973 mg, almost quantitative yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.67 (s, 3H, 3-CH_3), 2.57 (s, 3H, 4-CH_3), 3.40-3.73 (q, AB, 2H, J_{AB}=18.0 Hz, 5-H), 4.58-4.73 (q, AB, 2H, $J_{AB}\simeq$17.0 Hz, CH_2CO$_2$H), 7.27-7.45 (m, 5H, aromatic H), 9.30-10.10 (br s, 1H, CO$_2$*H*); ^{13}C NMR (100 MHz, $CDCl_3$) δ 24.3 (3-*C*H$_3$), 38.2 (4-*C*H$_3$), 40.1 (*C*H$_2$CO$_2$H), 55.0 (5-C), 68.1 (3-C), 126.1, 128.4, 129.0 (2,3,4,5,6-aromatic C), 140.4 (1-aromatic C), 170.3 (*C*O$_2$H), 173.3, 173.6 (2,6-C); ESI$^+$ MS: m/z 277.1 [M+H]$^+$.

4.1.32. 4-Methyl-2,6-Dioxo-3-phenyl-3-propyl-1-piperazineacetic acid **58**

Prepared from benzyl ester **53** (2.6 g, 6.59 mmol) by catalytic hydrogenolysis (H_2/10% Pd-C, 312 mg) in abs EtOH (165 mL) as described for compound **33**. The obtained white foamy solid strongly binds the hydrogenation solvent. Removal of the entrapped solvent as in **33** gave the title compound **58** as an off-white gum (1.98 g, 99%). The hydrochloride salt (**58·HCl**) was prepared by the same way described for **33·HCl**, and obtained as a white solid (decomposed gradually above 85 °C): ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.81 (t, 3H, J=7.3 Hz, CH_3(CH$_2$)$_2$), 1.03-1.28 (dm, 2H, CH$_3$CH_2CH$_2$), 1.82-1.93 (m, 1H, CH$_3$CH$_2$CHH), 2.08-2.21 (m, 1H, CH$_3$CH$_2$CHH), 2.38 (s, 3H, $^+$NHCH_3), 3.32-3.61 (q, AB, 2H, J_{AB}=18.2 Hz, 5-H), 3.66-4.35 (br s, 2H, $^+$NHCH$_3$, CO$_2H$), 4.41 (s, 2H, CH_2CO$_2$H), 7.30-7.43 (m, 5H, aromatic H); ^{13}C NMR (50 MHz, DMSO-d_6) δ 14.1 (CH$_3$(CH$_2$)$_2$), 16.4 (CH$_3$CH$_2$CH$_2$), 36.9 ($^+$NHCH$_3$), 37.5 (CH$_3$CH$_2$CH$_2$), 40.3 (CH$_2$CO$_2$H), 53.8 (5-C), 69.9 (3-C), 127.1, 128.1, 128.4 (2,3,4,5,6-aromatic C), 136.0 (1-aromatic C), 168.9 (CO$_2$H), 169.3, 173.0 (2,6-C). Anal. Calcd for $C_{16}H_{21}ClN_2O_4$: C, 56.39; H, 6.21; N, 8.22; Found: C, 56.02; H, 5.99; N, 8.53.

4.1.33. 3-Butyl-4-methyl-2,6-dioxo-3-phenyl-1-piperazineacetic acid **59**

Benzyl ester **54** (1.92 g, 4.7 mmol) was subjected to catalytic hydrogenolysis (H_2/10% Pd-C, 230 mg) in abs EtOH (118 mL) as described in **33** to afford the title compound **59** as a glass solid (1.48 g, 99%): ^{1}H NMR (600 MHz, CDCl$_3$) δ 0.83 (t, 3H, $J\simeq$7.0 Hz, CH_3(CH$_2$)$_3$), 1.06-1.14 (m, 1H, CH$_3$CH$_2$CHHCH$_2$), 1.21-1.32 (m, 3H, CH$_3$CH_2CHHCH$_2$), 2.08-2.27 (dm, 2H, CH$_3$(CH$_2$)$_2$CH_2), 2.77 (s, 3H, 4-CH_3), 3.70-3.89 (q, AB, 2H, J_{AB}=17.9 Hz, 5-H), 4.66 (s, 2H, CH_2CO$_2$H), 5.20-7.20 (v br s, 1H, CO$_2H$), 7.33-7.53 (m, 5H, aromatic H); ^{13}C NMR (50 MHz, CDCl$_3$) δ 13.9 (CH$_3$(CH$_2$)$_3$), 23.0 (CH$_3$CH$_2$(CH$_2$)$_2$), 25.6 (CH$_3$CH$_2$CH$_2$CH$_2$), 35.9 (CH$_3$(CH$_2$)$_2$CH$_2$), 38.3 (4-CH$_3$), 40.6 (CH$_2$CO$_2$H), 54.2 (5-C), 72.1 (3-C), 127.2, 129.3 (2,3,4,5,6-aromatic C), 134.4 (1-aromatic C), 167.4 (CO$_2$H), 171.1, 171.6 (2,6-C); ESI$^+$ MS: m/z 319.2 [M+H]$^+$.

4.1.34. 3-(4-Fluorophenyl)-3,4-dimethyl-2,6-dioxo-1-piperazineacetic acid **60**

Prepared from benzyl ester **55** (1.75 g, 4.55 mmol) by catalytic hydrogenolysis (H_2/10% Pd-C, 210 mg) in abs EtOH (114 mL) as described for compound **33**. The obtained white foamy solid strongly binds the hydrogenation solvent. Removal of the entrapped solvent as in **33** gave the title compound **60** as a glass solid (1.33 g, almost quantitative yield): ^{1}H NMR (400 MHz, CDCl$_3$) δ 1.66 (s, 3H, 3-CH_3), 2.54 (s, 3H, 4-CH_3), 3.44-3.70 (q, AB, 2H, J_{AB}=18.1 Hz, 5-H), 4.58-4.73 (q, AB, 2H, $J_{AB}\simeq$17.1 Hz, CH_2CO$_2$H), 7.05 (t, 2H, $J\simeq$8.6 Hz, 3,5-aromatic H), 7.38-7.48 (q, 2H, J=5.2 Hz, 2,6-aromatic H), 9.98-10.22 (br s, 1H, CO$_2H$); ^{13}C NMR (50 MHz, CDCl$_3$) δ 23.7 (3-CH$_3$), 38.1 (4-CH$_3$), 40.1 (CH$_2$CO$_2$H), 54.7 (5-C), 67.5 (3-C), 115.6, 116.0 (d, $J_{C\text{-}F}$=21.3 Hz, 3,5-aromatic C), 128.0, 128.2 (d, $J_{C\text{-}F}$=7.9 Hz, 2,6-aromatic C), 136.1 (1-aromatic C), 160.0, 164.9 (d, $J_{C\text{-}F}$=246 Hz, 4-aromatic C), 170.1 (CO$_2$H), 172.8, 173.4 (2,6-C); ESI$^+$ MS: m/z 295.3 [M+H]$^+$.

4.1.35. 2-Phenyl-2-propylaminopropanamide **66**

Acetophenone **18** (3.5 g, 29 mmol) was subjected to the Strecker reaction with *n*-propylamine hydrochloride (2.87 g, 30 mmol) and sodium cyanide (1.47 g, 30 mmol) following the procedure described for the preparation of compound **23**. The obtained crude oily α-aminonitrile was then hydrated with H_2SO_4 97% (37 mL) as described in **23**. The resulting crude thick oil was purified by column chromatography eluting with AcOEt-*n*-hexane 1:4 to 1:0 to afford the title compound **66** as an off-yellow solid (2.7 g, 45% overall yield). An analytical sample was obtained as white crystals upon recrystallization of the product from Et$_2$O-*n*-pentane. Mp 56-57 °C. ^{1}H NMR (400 MHz, CDCl$_3$) δ 0.91 (t, 3H, J=7.4 Hz, HN(CH$_2$)$_2$CH_3), 1.40-1.54 (m, 2H, HNCH$_2$CH_2CH$_3$), 1.56-1.93 (v br s, 1H, HN(CH$_2$)$_2$CH$_3$), 1.72 (s, 3H, CH_3), 2.32-2.52 (sym m, 2H, HNCH_2CH$_2$CH$_3$), 6.50 (br s, 1H, CONHH), 7.01 (br s, 1H, CONHH), 7.20-7.37 (m, 3H, aromatic H), 7.51 (d, 2H, J=7.2 Hz, aromatic H); ^{13}C NMR (50 MHz, CDCl$_3$) δ 11.8 (HN(CH$_2$)$_2$CH$_3$), 22.6 (CH$_3$), 24.0 (HNCH$_2$CH$_2$CH$_3$), 45.3 (HNCH$_2$CH$_2$CH$_3$), 64.8 (Ph(CH$_3$)CNH), 126.2, 127.4, 128.5 (2,3,4,5,6-aromatic C), 143.0 (1-aromatic C), 178.3 (CONH$_2$). Anal. Calcd for $C_{12}H_{18}N_2O$: C, 69.87; H, 8.80; N, 13.58; Found: C, 70.14; H, 9.12; N, 13.61.

4.1.36. 2-Butylamino-2-phenylpropanamide **67**

Acetophenone **18** (3.5 g, 29 mmol) was subjected to the Strecker reaction with *n*-butylamine hydrochloride (3.29 g, 30 mmol) and sodium cyanide (1.47 g, 30 mmol) following the procedure described for the preparation

of compound **23**. The crude oily α-aminonitrile obtained was then hydrated with H_2SO_4 97% (37 mL). The resulting crude thick oil was purified by column chromatography eluting with AcOEt-*n*-hexane 1:3 to 1:0 to afford the title compound **67** as a white crystalline solid (3 g, 47% overall yield): mp 83-85 °C (Et_2O-*n*-pentane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.89 (t, 3H, *J*=7.2 Hz, $HN(CH_2)_3CH_3$), 1.10-1.62 (v br s, 1H, $HN(CH_2)_3CH_3$), 1.27-1.50 (dm, 4H, $HNCH_2CH_2CH_2CH_3$), 1.72 (s, 3H, CH_3), 2.36-2.53 (sym m, 2H, $HNCH_2(CH_2)_2CH_3$), 6.33 (s, 1H, CONHH), 7.01 (s, 1H, CONHH), 7.20-7.38 (m, 3H, aromatic H), 7.51 (d, 2H, *J*=7.2 Hz, aromatic H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 14.1 ($HN(CH_2)_3CH_3$), 20.5 ($HN(CH_2)_2CH_2CH_3$), 22.6 (CH_3), 33.1 ($HNCH_2CH_2CH_2CH_3$), 43.2 ($HNCH_2(CH_2)_2CH_3$), 64.9 ($Ph(CH_3)CNH$), 126.2, 127.4, 128.5 (2,3,4,5,6-aromatic C), 143.1 (1-aromatic C), 178.3 ($CONH_2$). Anal. Calcd for $C_{13}H_{20}N_2O$: C, 70.87; H, 9.15; N, 12.72; Found: C, 71.04; H, 9.31; N, 12.52.

4.1.37. N-[(1-Aminocarbonyl-1-phenyl)ethyl]-N-propylglycine ethyl ester **68**

Sodium bicarbonate (336 mg, 4 mmol) and ethyl bromoacetate (731 mg, 4.38 mmol) were added successively to a solution of the aminoamide derivative **66** (765 mg, 3.71 mmol) in dry DMF (46 mL), and the resulting mixture was heated at 40-43 °C for 6 days with stirring. The reaction mixture was then poured into ice-water (40 mL) and extracted with AcOEt (3 × 40 mL). The combined organic phase was washed once with brine (40 mL), dried (Na_2SO_4) and concentrated to dryness under reduced pressure. The remaining oily residue was chromatographed on silica gel column with AcOEt-*n*-hexane 1:2, as eluent, to afford the title compound **68** as a white crystalline solid (615 mg, 57%): mp 105-106 °C (Et_2O-*n*-pentane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.80 (t, 3H, *J*≃7.4 Hz, $N(CH_2)_2CH_3$), 1.26 (t, 3H, *J*=7.2 Hz, $CO_2CH_2CH_3$), 1.29-1.48 (m, 2H, $NCH_2CH_2CH_3$), 1.65 (s, 3H, CH_3), 2.23-2.50 (dm, 2H, $NCH_2CH_2CH_3$), 3.10-3.40 (q, AB, 2H, J_{AB}≃17.4 Hz, $NCH_2CO_2CH_2CH_3$), 4.11-4.22 (qd, 2H, J_1≃1.8 Hz, J_2≃7.0 Hz, $CO_2CH_2CH_3$), 5.60 (s, 1H, CONHH), 7.21-7.36 (m, 3H, aromatic H), 7.63 (d, 2H, *J*=7.6 Hz, aromatic H), 7.82 (s, 1H, CONHH); ^{13}C NMR (50 MHz, $CDCl_3$) δ 11.8 ($NCH_2CH_2CH_3$), 14.2 ($CO_2CH_2CH_3$) 14.8 (CH_3), 22.1 ($NCH_2CH_2CH_3$), 55.0 ($NCH_2CO_2CH_2CH_3$), 55.5 ($NCH_2CH_2CH_3$), 61.2 ($CO_2CH_2CH_3$), 72.4 ($Ph(CH_3)CNH$), 127.2, 127.7, 128.5 (2,3,4,5,6-aromatic C), 141.7 (1-aromatic C), 173.5 ($CO_2CH_2CH_3$), 176.8 ($CONH_2$). Anal. Calcd for $C_{16}H_{24}N_2O_3$: C, 65.73; H, 8.27; N, 9.58; Found: C, 65.92; H, 8.65; N, 9.54.

4.1.38. N-[(1-Aminocarbonyl-1-phenyl)ethyl]-N-butylglycine ethyl ester **69**

Prepared from the aminoamide derivative **67** (881 mg, 4 mmol) by the same procedure described for compound **68**. The resulting crude thick oil was chromatographed on silica gel column with AcOEt-*n*-hexane 1:3, as eluent, to afford the title compound **69** as a white solid (573 mg, 47%): mp 108-110 °C (Et_2O-*n*-pentane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.80 (t, 3H, *J*=7.2 Hz, $N(CH_2)_3CH_3$), 1.08-1.46 (m, 4H, $NCH_2CH_2CH_2CH_3$), 1.27 (t, 3H, *J*≃7.0 Hz, $CO_2CH_2CH_3$), 1.64 (s, 3H, CH_3), 2.27-2.35 (m, 1H, $NCHH(CH_2)_2CH_3$), 2.42-2.55 (m, 1H, $NCHH(CH_2)_2CH_3$), 3.08-3.39 (q, AB, 2H, J_{AB}=17.2 Hz, $NCH_2CO_2CH_2CH_3$), 4.08-4.26 (sym m, 2H, $CO_2CH_2CH_3$), 5.64 (br s, 1H, CONHH), 7.20-7.38 (m, 3H, aromatic H), 7.63 (d, 2H, *J*=8.0 Hz, aromatic H), 7.82 (s, 1H, CONHH); ^{13}C NMR (50 MHz, $CDCl_3$) δ 14.1 ($N(CH_2)_3CH_3$), 14.2 ($CO_2CH_2CH_3$), 14.8 (CH_3), 20.5 ($N(CH_2)_2CH_2CH_3$), 31.2 ($NCH_2CH_2CH_2CH_3$) 53.3 ($NCH_2(CH_2)_2CH_3$), 55.0 ($NCH_2CO_2CH_2CH_3$), 61.1 ($CO_2CH_2CH_3$), 72.4 ($Ph(CH_3)CNH$), 127.2, 127.6, 128.4 (2,3,4,5,6-aromatic C), 141.8 (1-aromatic C), 173.6 ($CO_2CH_2CH_3$), 176.9 ($CONH_2$). Anal. Calcd for $C_{17}H_{26}N_2O_3$: C, 66.64; H, 8.55; N, 9.14; Found: C, 66.95; H, 8.81; N, 8.92.

4.1.39. 3-Methyl-2,6-dioxo-3-phenyl-4-propyl-1-piperazineacetic acid benzyl ester **70**

The amide-ester derivative **68** (710 mg, 2.43 mmol) was converted to the title benzyl ester **70** following the procedure described for the preparation of compound **28**. Purification of the crude product (yellowish oil) by column chromatography on silica gel (AcOEt-*n*-hexane 1:5) gave 850 mg (89%) of colourless viscous oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.82 (t, 3H, *J*≃7.4 Hz, $N(CH_2)_2CH_3$), 1.32-1.48 (m, 2H, $NCH_2CH_2CH_3$), 1.56 (s, 3H, 3-CH_3), 2.38-2.49 (m, 1H, $NCHHCH_2CH_3$), 2.55-2.65 (m, 1H, $NCHHCH_2CH_3$), 3.40-3.57 (q, AB, 2H, J_{AB}≃18.2 Hz, 5-H), 4.47-4.65 (q, AB, 2H, J_{AB}=16.8 Hz, $CH_2CO_2CH_2Ph$), 5.10 (s, 2H, CO_2CH_2Ph), 7.15-7.40 (complex m, 10H, aromatic H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 11.6 ($N(CH_2)_2CH_3$), 21.5 ($NCH_2CH_2CH_3$), 23.5 (3-CH_3), 40.4 ($CH_2CO_2CH_2Ph$), 50.5 ($NCH_2CH_2CH_3$), 51.1 (5-C), 67.4 (CO_2CH_2Ph), 68.0 (3-C), 126.2, 128.1, 128.4, 128.6, 128.7, 128.9, 129.3, 129.4, 135.2, 141.4

(aromatic C), 167.7 (CO_2CH_2Ph), 170.8, 173.9 (2,6-C); HRMS (ESI): $[M+H]^+$ calcd for $C_{23}H_{26}N_2O_4$, 395.1971, found 395.1968.

4.1.40. 4-Butyl-3-methyl-2,6-dioxo-3-phenyl-1-piperazineacetic acid benzyl ester **71**

The amide-ester derivative **69** (560 mg, 1.83 mmol) was converted to the title benzyl ester **71** following the procedure described for the preparation of compound **28**. Purification of the crude product (off-yellow thick oil) by column chromatography on silica gel (AcOEt-*n*-hexane 1:6) gave 606 mg (81%) of colourless viscous oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.90 (t, 3H, J=7.2 Hz, $N(CH_2)_3CH_3$), 1.21-1.53 (complex m, 4H, $NCH_2CH_2CH_2CH_3$), 1.65 (s, 3H, 3-CH_3), 2.46-2.58 (m, 1H, $NCH(CH_2)_2CH_3$), 2.68-2.81 (m, 1H, $NCH(CH_2)_2CH_3$), 3.48-3.68 (q, AB, 2H, J_{AB}=18.0 Hz, 5-H), 4.53-4.76 (q, AB, 2H, J_{AB}=16.8 Hz, $CH_2CO_2CH_2Ph$), 5.19 (s, 2H, CO_2CH_2Ph), 7.20-7.50 (complex m, 10H, aromatic H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 14.0 ($N(CH_2)_3CH_3$), 20.2 ($N(CH_2)_2CH_2CH_3$), 23.5 (3-CH_3), 30.5 ($NCH_2CH_2CH_2CH_3$), 40.5 ($CH_2CO_2CH_2Ph$), 48.4 ($NCH_2(CH_2)_2CH_3$), 51.1 (5-C), 67.4 (CO_2CH_2Ph), 68.0 (3-C), 126.2, 128.2, 128.4, 128.6, 128.7, 128.9, 135.3, 141.4 (aromatic C), 167.7 (CO_2CH_2Ph), 170.8, 173.9 (2,6-C); HRMS (ESI): $[M+H]^+$ calcd for $C_{24}H_{28}N_2O_4$, 409.2127, found 409.2150.

4.1.41. 3-Methyl-2,6-dioxo-3-phenyl-4-propyl-1-piperazineacetic acid **72**

Benzyl ester **70** (1.25 g, 3.17 mmol) was subjected to catalytic hydrogenolysis (H_2/10% Pd-C, 150 mg) in abs EtOH (143 mL) as described in **33** to give a colourless glass solid. Trituration of this product material with Et_2O gave the title compound **72** as a white crystalline solid (900 mg, 93%). A recrystallized sample had mp 152-154 °C (from Et_2O). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.92 (t, 3H, J=7.2 Hz, $N(CH_2)_2CH_3$), 1.44-1.60 (sym m, 2H, $NCH_2CH_2CH_3$), 1.66 (s, 3H, 3-CH_3), 2.49-2.63 (m, 1H, $NCHHCH_2CH_3$), 2.68-2.80 (m, 1H, $NCHHCH_2CH_3$), 3.47-3.70 (q, AB, 2H, J_{AB}=18.4 Hz, 5-H), 4.53-4.73 (q, AB, 2H, $J_{AB}\simeq$17.0 Hz, CH_2CO_2H), 7.20-7.50 (complex m, 5H, aromatic H), 9.61-10.53 (br s, 1H, CO_2H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 11.6 ($N(CH_2)_2CH_3$), 21.5 ($NCH_2CH_2CH_3$), 23.6 (3-CH_3), 40.1 (CH_2CO_2H), 50.7 ($NCH_2CH_2CH_3$), 51.1 (5-C), 68.0 (3-C), 126.2, 128.2, 128.9 (2,3,4,5,6-aromatic C), 141.3 (1-aromatic C), 170.8 (CO_2H), 173.5, 173.9 (2,6-C). Anal. Calcd for $C_{16}H_{20}N_2O_4$: C, 63.14; H, 6.62; N, 9.21; Found: C, 63.43; H, 6.72; N, 9.52.

4.1.42. 4-Butyl-3-methyl-2,6-dioxo-3-phenyl-1-piperazineacetic acid **73**

Benzyl ester **71** (600 mg, 1.47 mmol) was subjected to catalytic hydrogenolysis (H_2/10% Pd-C, 72 mg) in abs EtOH (66 mL) as described in **33** to give a colourless glass solid. Trituration of this product material with a diethyl ether-*n*-pentane 1:1 mixture afforded the title compound **73** as a white crystalline solid (460 mg, 98%). A specimen was recrystallized from Et_2O-*n*-pentane to give an analytical sample which had mp 102-103 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.91 (t, 3H, J=7.2 Hz, $N(CH_2)_3CH_3$), 1.25-1.57 (complex m, 4H, $NCH_2CH_2CH_2CH_3$), 1.67 (s, 3H, 3-CH_3), 2.50-2.63 (m, 1H, $NCHH(CH_2)_2CH_3$), 2.75-2.85 (m, 1H, $NCH(CH_2)_2CH_3$), 3.51-3.68 (q, AB, 2H, J_{AB}=18.0 Hz, 5-H), 4.53-4.72 (q, AB, 2H, $J_{AB}\simeq$17.0 Hz, CH_2CO_2H), 7.26-7.48 (m, 5H, aromatic H), 9.80-11.1 (v br s, 1H, CO_2H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 14.0 ($N(CH_2)_3CH_3$), 20.3 ($N(CH_2)_2CH_2CH_3$), 23.6 (3-CH_3), 30.4 ($NCH_2CH_2CH_2CH_3$), 40.1 (CH_2CO_2H), 48.6 ($NCH_2(CH_2)_2CH_3$), 51.1 (5-C), 68.1 (3-C), 126.2, 128.2, 128.9 (2,3,4,5,6-aromatic C), 141.3 (1-aromatic C), 170.8 (CO_2H), 173.6, 173.9 (2,6-C); Anal. Calcd for $C_{17}H_{22}N_2O_4$: C, 64.13; H, 6.97; N, 8.80; Found: C, 63.82; H, 7.12; N, 9.03.

4.1.43. N-Hydroxy-3-methyl-2,6-dioxo-3-phenyl-1-piperazineacetamide **6**

1,1'-Carbonyldiimidazol (409 mg, 2.52 mmol) was added to a solution of carboxylic acid **33** (550 mg, 2.1 mmol) in dry THF (42 mL), and the mixture was allowed to stir at 28 °C for 1 h under argon. Then, *O*-benzylhydroxylamine hydrochloride (402 mg, 2.52 mmol) was added followed by triethylamine (510 mg, 5.04 mmol). After the mixture was stirred for 24 h at 28 °C and 1 h at 45 °C under argon, the solvent was evaporated under reduced pressure. Water (50 mL) was added to the residue, and the mixture was extracted with ethyl acetate (3 × 50 mL). The combined organic phase was washed with brine (2 × 50 mL), dried (Na_2SO_4) and evaporated to dryness *in vacuo*. The viscous oily residue was purified by column chromatography on silica gel with AcOEt-*n*-hexane 2:1, as eluent, to afford the *N*-benzyloxy precursor **37** as a white foamy solid, which strongly binds the elution solvents. Removal of the entrapped solvents upon drying at 62-64 °C under high

vacuum (10^{-2} mmHg) in an Abderhalden apparatus gave **37** as a glass solid (572 mg, 74%). This compound appears in the ^{1}H and ^{13}C NMR spectra as a mixture of *E*/*Z* conformers (not assigned). ^{1}H NMR (600 MHz, $CDCl_3$, 283 K) δ 1.59, 1.63 (s + s, 3H, C*H*$_3$), 2.26-2.74 (br s, 1H, 4-H), 3.36-3.50 (q, 1H, *J*=18.5 Hz, 5-H), 3.59-3.71 (q, 1H, *J*=18.5 Hz, 5-H), 4.30-4.43 (q, AB, 1.14H, $J_{AB}\simeq$15.1 Hz, C*H*$_2$CONHOCH$_2$Ph), 4.70 (s, 0.94H, C*H*$_2$CONHOCH$_2$Ph), 4.87, 4.91 (s + s, 2H, CONHOC*H*$_2$Ph), 7.28-7.50 (complex m, 10H, aromatic H), 8.59 (s, 0.48H, CON*H*OCH$_2$Ph), 9.37 (s, 0.57H, CON*H*OCH$_2$Ph); ^{13}C NMR (150 MHz, $CDCl_3$, 283 K) δ 28.7 (*C*H$_3$), 39.4 (*C*H$_2$CONHOCH$_2$Ph), 45.9 (5-C), 62.5 (3-C), 78.2, 79.5 (CONHO*C*H$_2$Ph), 125.6, 125.7, 128.1, 128.5, 128.7, 128.8, 128.9, 129.1, 129.3, 129.4, 134.0, 134.9, 138.7 (aromatic C), 165.2, 170.3 (*C*ONHOCH$_2$Ph), 171.8, 173.8 (2,6-C); ESI$^+$MS: m/z 368.2 [M+H]$^+$.

A solution of *O*-benzyl hydroxamate **37** (570 mg, 1.55 mmol) in abs EtOH (70 mL) was hydrogenated over 10% Pd-C (68 mg) at room temperature under 50 psi of hydrogen. After 3 h the catalyst was filtered off, washed with EtOH (3 × 15 mL), and the combined filtrates were evaporated to dryness under reduced pressure. The residual material (off-white foamy solid) was chromatographed on silica gel column with AcOEt-MeOH 15:1, as eluent, to afford the title compound **6** as a white foamy solid, which strongly binds the aforementioned solvents. Removal of the entrapped solvents upon drying at 62-64 °C under high vacuum (10^{-2} mmHg) in an Abderhalden apparatus gave **6** as a slightly off-yellow crystalline solid (350 mg, 81%): mp 80-83 °C (dec); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.51 (s, 3H, C*H*$_3$), 3.07-3.19 (q, 1H, *J*=10.7 Hz, 5-H), 3.53-3.64 (dd, 1H, *J*=4.1, 18.3 Hz, 5-H), 3.79-3.90 (dd, 1H, *J*=4.0, 10.6 Hz, 4-H), 4.19-4.32 (q, AB, 1.5H, J_{AB}=15.6 Hz, C*H*$_2$CONHOH, *E*-isomer), 4.49-4.61 (q, AB, 0.46H, $J_{AB}\approx$17.4 Hz, C*H*$_2$CONHOH, *Z*-isomer), 7.27-7.52 (complex m, 5H, aromatic H), 8.93 (s, 0.74H, CONHO*H*, *E*-isomer), 9.34 (s, 0.2H, CONHO*H*, *Z*-isomer), 10.21 (s, 0.2H, CON*H*OH, *Z*-isomer), 10.65 (s, 0.75H, CON*H*OH, *E*-isomer); ^{13}C NMR (100 MHz, DMSO-d_6) δ 28.2 (*C*H$_3$), 39.0 (*C*H$_2$CONHOH, *E*-isomer), 39.4 (*C*H$_2$CONHOH, *Z*-isomer), 45.8 (5-C), 62.1 (3-C), 126.0, 126.4, 126.6, 127.7, 128.1, 128.5, 128.6 (2,3,4,5,6-aromatic C), 140.2, 140.3 (1-aromatic C), 163.9 (*C*ONHOH, *E*-isomer), 169.3 (*C*ONHOH, *Z*-isomer), 171.5, 173.7, 173.8 (2,6-C); HRMS (APCI$^+$): [M+H]$^+$ calcd for $C_{13}H_{15}N_3O_4$, 278.1135, found 278.1136. The hydrochloride salt (**6·HCl**) was prepared by treating a solution of **6** in AcOEt-Et_2O 2:3 with saturated solution of HCl in Et_2O under ice cooling. The solvents were then evaporated under reduced pressure, and the white solid was triturated with Et_2O, filtered and dried in vacuo. Mp 141-145 °C (dec) (slightly hygroscopic). Anal. Calcd for $C_{13}H_{16}ClN_3O_4$: C, 49.77; H, 5.14; N, 13.39; Found: C, 49.38; H, 5.52; N, 13.03.

4.1.44. N-Hydroxy-2,6-dioxo-3-phenyl-3-propyl-1-piperazineacetamide **7**

The *N*-benzyloxy precursor **38** was prepared from carboxylic acid **34** (700 mg, 2.41 mmol) following the procedure described for the preparation of compound **37** (precursor for **6**). The crude viscous oil was purified by column chromatography on silica gel with AcOEt-*n*-hexane 2:3, as eluent, to afford **38** as a glass solid (675 mg, 71%). This compound appears in the ^{1}H and ^{13}C NMR spectra as a mixture of *E*/*Z* conformers (not assigned). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.75 (t, 3H, *J*=7.2 Hz, C*H*$_3$(CH$_2$)$_2$), 1.10-1.28 (m, 2H, CH$_3$C*H*$_2$CH$_2$), 1.72-2.02 (dm, 2H, CH$_3$CH$_2$C*H*$_2$), 2.43 (s, 1H, 4-H), 3.28-3.69 (dm, 2H, 5-H), 4.14-4.39 (m, 1H, C*H*$_2$CONHOCH$_2$Ph), 4.60, 4.65 (s + s, 0.95H, C*H*$_2$CONHOCH$_2$Ph), 4.78 (s, 2H, CONHOC*H*$_2$Ph), 7.10-7.45 (m, 10H, aromatic H), 8.71 (s, 0.3H, CON*H*OCH$_2$Ph), 9.46 (s, 0.4H, CON*H*OCH$_2$Ph); ^{13}C NMR (50 MHz, $CDCl_3$) δ 14.3 (*C*H$_3$(CH$_2$)$_2$), 17.2 (CH$_3$*C*H$_2$CH$_2$), 39.5 (*C*H$_2$CONHOCH$_2$Ph), 43.9 (CH$_3$CH$_2$*C*H$_2$), 45.9 (5-C), 65.7 (3-C), 78.6, 79.5 (CONHO*C*H$_2$Ph), 126.2, 128.1, 128.7, 128.8, 129.3, 135.1, 137.8 (aromatic C), 165.4, 170.5 (*C*ONHOCH$_2$Ph), 171.8, 173.4 (2,6-C); EI MS: m/z 396.2 ([M+H]$^+$, 9), 395.1 ([M]$^+$, 27), 352.1 ([M-CH$_2$CH$_2$CH$_3$]$^+$, 25), 305.1 ([M+H-CH$_2$Ph]$^+$, 11), 304.0 ([M-CH$_2$Ph]$^+$, 67), 217.0 (73), 160.0 (83), 90.9 (100).

Compound **38** (1.2 g, 3.03 mmol) was subjected to catalytic hydrogenation (H_2/10% Pd-C, 144 mg), in abs EtOH (136 mL) as described for the preparation of compound **6** from **37**. The hydrogenation product (off-white foamy solid) was chromatographed on silica gel column eluting first with AcOEt-*n*-hexane 1:2 and then AcOEt to afford the title compound **7** as a white foamy solid. This material gave white crystals upon dissolving in Et_2O (15 mL) and subsequent evaporation of the solvent twice. (700 mg, 76%): mp 148-150 °C (dec) (MeOH-Et_2O); ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.78 (t, 3H, *J*=6.3, 7.4 Hz, C*H*$_3$(CH$_2$)$_2$), 1.12-1.36 (m, 2H, CH$_3$C*H*$_2$CH$_2$), 1.70-1.81 (td, 1H,

J≈4.5 Hz, *J*=11.9, 13.4 Hz, CH_3CH_2CHH), 1.82-1.93 (td, 1H, *J*=2.6-4.0 Hz, *J*=11.9, 13.1 Hz, CH_3CH_2CHH), 3.16-3.27 (q, 1H, *J*=10.1 Hz, 5-H), 3.56-3.66 (dd, 1H, *J*=3.7, 18.1 Hz, 5-H), 3.69-3.78 (dd, 1H, *J*≃2.8, 9.8 Hz, 4-H), 4.15-4.30 (q, AB, 1.53H, J_{AB}=15.5 Hz, $CH_2CONHOH$, *E*-isomer), 4.45-4.59 (q, AB, 0.41H, J_{AB}=16.8 Hz, $CH_2CONHOH$, *Z*-isomer), 7.25-7.51 (m, 5H, aromatic H), 8.92 (s, 0.7H, CONHO*H*, *E*-isomer), 9.33 (s, 0.2H, CONHO*H*, *Z*-isomer), 10.20 (s, 0.2H, CON*H*OH, *Z*-isomer), 10.63 (s, 0.7H, CON*H*OH, *E*-isomer); ^{13}C NMR (50 MHz, DMSO-d_6) δ 14.2 ($CH_3(CH_2)_2$), 16.7 ($CH_3CH_2CH_2$), 38.9 ($CH_2CONHOH$), 43.4 ($CH_3CH_2CH_2$), 45.7 (5-C), 65.0 (3-C), 126.2, 127.6, 128.5 (2,3,4,5,6-aromatic C), 138.7 (1-aromatic C), 163.8 (*C*ONHOH, *E*-isomer), 169.2 (*C*ONHOH, *Z*-isomer), 171.3, 173.1 (2,6-C); ESI$^+$ MS: m/z 306.5 [M+H]. Anal. Calcd for $C_{15}H_{19}N_3O_4$: C, 59.01; H, 6.27; N, 13.76; Found: C, 58.87; H, 6.30; N, 13.92. The hydrochloride salt (**7·HCl**) was prepared by treating a solution of **7** in MeOH-Et_2O 1:15 with saturated solution of HCl in Et_2O under ice cooling, and was fully precipitated by adding Et_2O. The white solid was collected by vacuum filtration, triturated with Et_2O, and dried in vacuo (decomposed gradually above 115 °C). Anal. Calcd for $C_{15}H_{20}ClN_3O_4 \cdot 0.15\ Et_2O$: C, 53.09; H, 6.14; N, 11.90; Found: C, 52.80; H, 6.21; N, 11.62.

4.1.45. 3-Butyl-N-hydroxy-2,6-dioxo-3-phenyl-1-piperazineacetamide **8**

The *N*-benzyloxy precursor **39** was prepared from carboxylic acid **35** (1 g, 3.29 mmol) following the procedure described for the preparation of compound **37** (precursor for **6**). The crude oil was purified by flash column chromatography eluting with AcOEt-*n*-hexane 1:1 to afford **39** as an off-yellow glass oil (870 mg, 64%). This compound appears in the ^{1}H and ^{13}C NMR spectra as a mixture of *E*/*Z* conformers (not assigned). ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.77 (t, 3H, *J*=5.6 Hz, $CH_3(CH_2)_3$), 1.06-1.30 (m, 4H, $CH_3CH_2CH_2CH_2$), 1.75-2.06 (dm, 2H, $CH_3(CH_2)_2CH_2$), 2.27-2.49 (br s, 1H, 4-H), 3.44 (d, 1H, *J*=17.0 Hz, 5-H), 3.62 (d, 1H, *J*=18.2 Hz, 5-H), 4.28 (br d, 0.76H, *J*=26.8 Hz, $CH_2CONHOCH_2Ph$), 4.63, 4.68 (s + s, 1.47H, $CH_2CONHOCH_2Ph$), 4.83 (s, 2H, $CONHOCH_2Ph$), 7.16-7.44 (complex m, 10H, aromatic H), 8.32 (s, 0.26H, CON*H*OCH_2Ph), 8.87 (s, 0.23H, CON*H*OCH_2Ph); ^{13}C NMR (50 MHz, $CDCl_3$) δ 13.9 ($CH_3(CH_2)_3$), 22.9 ($CH_3CH_2(CH_2)_2$), 25.9 ($CH_3CH_2CH_2CH_2$), 39.5 ($CH_2CONHOCH_2Ph$), 41.6 ($CH_3(CH_2)_2CH_2$), 46.0 (5-C), 65.7 (3-C), 78.4, 79.6 ($CONHOCH_2Ph$), 126.3, 128.2, 128.8, 128.9, 129.3, 129.4, 135.1, 137.9 (aromatic C), 160.0 (*C*ONHOCH_2Ph), 171.7, 173.4 (2,6-C); EI MS: m/z 410.1 ([M+H]$^+$, 17), 409.1 ([M]$^+$, 55), 352.1 ([M-$(CH_2)_3CH_3$]$^+$, 59), 318.1 ([M-CH_2Ph]$^+$, 100), 302.1 ([M-OCH_2Ph]$^+$, 47).

Compound **39** (900 mg, 2.2 mmol) was subjected to catalytic hydrogenation (H_2/10% Pd-C, 108 mg) in abs EtOH (98 mL) as described for the preparation of compound **6** from **37**. The crude hydrogenation product (glass oil) was chromatographed on silica gel column with AcOEt, as eluent, to afford the title compound **8** as a white crystalline solid (530 mg, 75%): mp 158-160 °C (dec) (MeOH-Et_2O); ^{1}H NMR (600 MHz, DMSO-d_6) δ 0.80 (t, 3H, *J*=7.0 Hz, $CH_3(CH_2)_3$), 1.12-1.29 (complex m, 4H, $CH_3CH_2CH_2CH_2$), 1.75-1.95 (dm, 2H, $CH_3(CH_2)_2CH_2$), 3.18-3.26 (q, 1H, *J*=10.0 Hz, 4-H), 3.58-3.64 (dd, 1H, *J*=4.1, 18.0 Hz, 5-H), 3.69-3.75 (dd, 1H, *J*=4.1, 10.1 Hz, 5-H), 4.17-4.28 (q, AB, 1.6H, J_{AB}=15.5 Hz, $CH_2CONHOH$, *E*-isomer), 4.47-4.57 (q, AB, 0.4H, J_{AB}=16.7 Hz, $CH_2CONHOH$, *Z*-isomer), 7.28-7.48 (m, 5H, aromatic H), 8.89 (s, 0.7H, CONHO*H*, *E*-isomer), 9.30 (s, 0.2H, CONHO*H*, *Z*-isomer), 10.17 (s, 0.2H, CON*H*OH, *Z*-isomer), 10.60 (s, 0.7H, CON*H*OH, *E*-isomer); ^{13}C NMR (50 MHz, DMSO-d_6) δ 13.9 ($CH_3(CH_2)_3$), 22.4 ($CH_3CH_2(CH_2)_2$), 25.5 ($CH_3CH_2CH_2CH_2$), 38.9 ($CH_2CONHOCH_2Ph$), 41.0 ($CH_3(CH_2)_2CH_2$), 45.7 (5-C), 65.0 (3-C), 126.2, 127.6, 128.5 (2,3,4,5,6-aromatic C), 138.8 (1-aromatic C), 163.8 (*C*ONHOH, *E*-isomer), 169.2 (*C*ONHOH, *Z*-isomer), 171.3, 173.1 (2,6-C); ESI$^+$ MS: m/z 342.3 [M+Na]$^+$. Anal. Calcd for $C_{16}H_{21}N_3O_4$: C, 60.17; H, 6.63; N, 13.16; Found: C, 60.25; H, 6.70; N, 12.98. The hydrochloride salt (**8·HCl**) was prepared as described for **7·HCl** (decomposed gradually above 100 °C). Anal. Calcd for $C_{16}H_{22}ClN_3O_4$: C, 54.01; H, 6.23; N, 11.81; Found: C, 53.63; H, 5.92; N, 11.54.

4.1.46. 3-(4-Fluorophenyl)-N-hydroxy-3-methyl-2,6-dioxo-1-piperazineacetamide **9**

A stirred solution of carboxylic acid **36** (750 mg, 2.68 mmol) and 1,1'-carbonyldiimidazol (522 mg, 3.22 mmol) in dry THF (53 mL) was heated at 55 °C for 1 h under argon. Then, *O*-benzylhydroxylamine hydrochloride (514 mg, 3.22 mmol) was added followed by triethylamine (652 mg, 6.44 mmol), and the mixture was stirred at 55 °C for 25 h under argon. The reaction was then worked up in the same way described in **37**. The resulting oily residue was purified by column chromatography eluting first with AcOEt-*n*-hexane 1:2 and then 1:1 to afford the

corresponding *N*-benzyloxy precursor **40** as a white foamy solid, which strongly binds the elution solvents. Removal of the entrapped solvents as in **37** gave **40** as a glass solid (632 mg, 61%): This compound appears in the ^{1}H and ^{13}C NMR spectra as a mixture of *E*/*Z* conformers (not assigned). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.57 (s, 3H, C*H*$_3$), 2.36-2.62 (br s, 1H, 4-H), 3.38 (t, 1H, *J*≃18.4 Hz, 5-H), 3.64 (t like, 1H, *J*=16.4, 17.8 Hz, 5-H), 4.23-4.44 (q, AB, 1H, J_{AB}≃14.8 Hz, C*H*$_2$CONHOCH$_2$Ph), 4.68 (s, 0.94H, C*H*$_2$CONHOCH$_2$Ph), 4.86, 4.90 (s + s, 2H, CONHOC*H*$_2$Ph), 7.04 (t, 2H, *J*=8.4 Hz, 3,5-H for 4-FC_6H_4), 7.15-7.55 (complex m, 7H, C_6H_5, 2,6-H for 4-FC_6H_4), 8.60 (br s, 0.4H, CON*H*OCH$_2$Ph), 9.30 (br s, 0.5H, CON*H*OCH$_2$Ph); ^{13}C NMR (50 MHz, $CDCl_3$) δ 29.0 (*C*H$_3$), 39.6 (*C*H$_2$CONHOCH$_2$Ph), 46.0 (5-C), 62.3 (3-C), 78.5, 79.7 (CONHO*C*H$_2$Ph), 115.7, 116.1 (d, $J_{C\text{-}F}$=21.3 Hz, 3,5-C for 4-FC_6H_4), 127.7, 127.9 (d, $J_{C\text{-}F}$=8.1 Hz, 2,6-C for 4-FC_6H_4), 128.8, 129.4 (2,3,4,5,6-C for C_6H_5), 134.7, 134.8 (1-C for 4-FC_6H_4 and C_6H_5), 160.1, 165.0 (d, $J_{C\text{-}F}$=246 Hz, 4-C for 4-FC_6H_4), 165.4, 170.5 (*C*ONHOCH$_2$Ph, weak signal intensities), 171.7, 173.8 (2,6-C); ESI$^+$ MS: m/z 386.3 $[M+H]^+$.

Compound **40** (1 g, 2.59 mmol) was subjected to catalytic hydrogenation (H_2/10% Pd-C, 120 mg), in abs EtOH (117 mL) as described for the preparation of compound **6** from **37**. The crude hydrogenation product (off-white foamy solid) was chromatographed on silica gel column with AcOEt, as eluent, to afford the title compound **9** as a white foamy solid, which gave white crystals upon trituration with Et_2O (720 mg, 94%): mp 154-158 °C (dec) (AcOEt-Et_2O); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.49 (s, 3H, C*H*$_3$), 3.02-3.19 (q, 1H, *J*=10.7 Hz, 5-H), 3.52-3.65 (dd, 1H, *J*=3.9, 18.3 Hz, 5-H), 3.80-3.95 (dd, 1H, *J*≃3.8, 10.5 Hz, 4-H), 4.24 (s, 1.5H, C*H*$_2$CONHOH, *E*-isomer), 4.54 (s, 0.5H, C*H*$_2$CONHOH, *Z*-isomer), 7.19 (t, 2H, *J*=8.7 Hz, 3,5-aromatic H), 7.51 (t like, 2H, *J*=5.6, 8.1 Hz, 2,6-aromatic H), 8.96 (s, 0.8H, CONHO*H*, *E*-isomer), 9.37 (s, 0.2H, CONHO*H*, *Z*-isomer), 10.25 (s, 0.2H, CON*H*OH, *Z*-isomer), 10.67 (s, 0.8H, CON*H*OH, *E*-isomer); ^{13}C NMR (50 MHz, DMSO-d_6) δ 28.3 (*C*H$_3$), 39.0 (*C*H$_2$CONHOH, *E*-isomer), 39.5 (*C*H$_2$CONHOH, *Z*-isomer), 45.6 (5-C), 61.7 (3-C), 115.2, 115.6 (d, $J_{C\text{-}F}$=21.1 Hz, 3,5-aromatic C), 128.2, 128.3 (d, $J_{C\text{-}F}$=7.5 Hz, 2,6-aromatic C), 136.4 (1-aromatic C), 159.2, 164.0 (d, $J_{C\text{-}F}$=242 Hz, 4-aromatic C), 163.9 (*C*ONHOH, *E*-isomer), 169.2 (*C*ONHOH, *Z*-isomer), 171.4, 173.6 (2,6-C); Anal. Calcd for $C_{13}H_{14}FN_3O_4$: C, 52.88; H, 4.78; N, 14.23; Found: C, 52.67; H, 4.88; N, 14.32. The hydrochloride salt was prepared by treating an ethyl acetate solution of **9** with saturated solution of HCl in Et_2O under ice cooling. The white precipitate was collected by vacuum filtration, triturated with Et_2O and dried (decomposed gradually above 120 °C). Anal. Calcd for $C_{13}H_{15}ClFN_3O_4$: C, 47.07; H, 4.56; N, 12.67; Found: C, 46.70; H, 4.48; N, 12.35.

4.1.47. N-Hydroxy-3-methyl-3-(4-nitrophenyl)-2,6-dioxo-1-piperazineacetamide Hydrochloride **10**

4-Methoxybenzyl ester **32** (1 g, 2.34 mmol) was taken in CH_2Cl_2 (26 mL) and treated with trifluoroacetic acid (4 mL). The mixture was left stirring at room temperature for 90 min, evaporated to dryness in vacuo, and dissolved in dry THF (46 mL). Triethylamine (474 mg, 4.68 mmol) and 1,1'-carbonyldiimidazol (456 mg, 2.81 mmol) were then added, and the mixture was stirred at 28 °C for 1 h under argon. After this time, *O*-(4-methoxybenzyl) hydroxylamine (430 mg, 2.81 mmol) was added, and the resulting mixture was stirred for 18 h at 28 °C and 7 h at 55 °C under argon. The solvent was removed under reduced pressure, and the residue was partitioned between ethyl acetate (100 mL) and brine (50 mL). The aqueous layer was extracted once more with ethyl acetate (50 mL), and the combined organic extracts were washed once with brine (50 mL), dried (Na_2SO_4) and evaporated in vacuo. The resulting yellow thick oil was purified by column chromatography eluting with AcOEt-*n*-hexane 1:1 to 4:1 to afford the *N*-(4-methoxybenzyloxy) precursor **41** as a yellowish foamy solid, which strongly binds the elution solvents. Removal of the entrapped solvents as in **37** gave **41** as a glass solid (500 mg, 48% yield over two steps). This compound appears in the ^{1}H and ^{13}C NMR spectra as a mixture of *E*/*Z* conformers (not assigned). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.64 (s, 3H, C*H*$_3$), 2.25-2.75 (br s, 1H, 4-H), 3.28-3.47 (m, 1H, 5-H), 3.72 (d, 1H, *J*=18.8 Hz, 5-H), 3.80 (s, 3H, OC*H*$_3$), 4.27-4.47 (q, AB, 0.9H, J_{AB}≃15.0 Hz, C*H*$_2$CONHOCH$_2$C$_6$H$_4$OCH$_3$-4), 4.66 (s, 1.1H, C*H*$_2$CONHOCH$_2$C$_6$H$_4$OCH$_3$-4), 4.82, 4.84 (s + s, 2H, CONHOC*H*$_2$C$_6$H$_4$OCH$_3$-4), 6.89 (s, 2H, 3,5-H for 4-$CH_3OC_6H_4$), 7.32 (d, 2H, *J*=8.5 Hz, 2,6-H for 4-$CH_3OC_6H_4$), 7.72 (d, 2H, *J*=7.4 Hz, 2,6-H for 4-$NO_2C_6H_4$), 8.21 (d, 2H, *J*=8.8 Hz, 3,5-H for 4-$NO_2C_6H_4$), 8.44 (s, 0.43H, CON*H*OCH$_2$C$_6$H$_4$OCH$_3$-4), 9.15 (s, 0.4H, CON*H*OCH$_2$C$_6$H$_4$OCH$_3$-4); ^{13}C NMR (50 MHz, $CDCl_3$) δ 29.0 (3-*C*H$_3$), 39.7 (*C*H$_2$CONHOCH$_2$C$_6$H$_4$OCH$_3$-4), 46.0 (5-C), 55.5 (O*C*H$_3$), 62.7 (3-C),

78.2, 79.4 (CONHO*C*$H_2C_6H_4OCH_3$-4), 114.3, 124.3, 127.3, 131.3, 146.5, 147.9, 160.4 (aromatic C), 164.9, 170.1 (*C*ONHO$CH_2C_6H_4OCH_3$-4), 171.0, 173.0 (2,6-C); ESI$^+$ MS: m/z 443.6 $[M+H]^+$.

A solution of compound **41** (500 mg, 1.13 mmol) and trifluoroacetic acid (8.3 mL) in CH_2Cl_2 (32 mL) was stirred for 10 min, and subsequently treated dropwise with Et_3SiH (830 µL) via syringe. After 45 min of stirring at room temperature, the color changed from ruby to pale yellow, and the mixture was evaporated to dryness under reduced pressure. A slurry of sodium chloride in water (3 mL) was added to the residue, and the mixture was treated slowly with solid Na_2CO_3 to pH 8-9. This thick mixture was then washed five times with ethyl acetate (15 mL) under vigorous stirring, and the combined washings were dried (Na_2SO_4) and concentrated to dryness in vacuo. The resulting oily residue was chromatographed on silica gel column with AcOEt, as eluent, to afford the free base **10** as a yellowish foamy solid (HRMS (ESI): $[M+H]^+$ calcd for $C_{13}H_{14}N_4O_6$, 323.0992, found 323.0994). This free base material was dissolved in AcOEt-Et_2O 1:1 and treated with saturated solution of HCl in Et_2O under ice cooling. The resulting precipitate was collected by vacuum filtration, triturated with Et_2O, and dried in vacuo to give 256 mg (63%) of the title compound **10** as an off-white hygroscopic solid (decomposed gradually above 160 °C); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.62 (s, 3H, CH_3), 3.28 (d, 1H, *J*=18.4 Hz, 5-H), 3.76 (d, 1H, *J*=18.4 Hz, 5-H), 4.27 (s, 1.5H, CH_2CONHOH, *E*-isomer), 4.55 (s, 0.5H, CH_2CONHOH, *Z*-isomer), 4.19-5.30 (v br s, NH_2^+, CONHO*H*, under DMSO water peak), 7.82 (d, 2H, *J*=8.4 Hz, 2,6-aromatic H), 8.24 (d, 2H, *J*=8.8 Hz, 3,5-aromatic H), 10.31 (s, 0.2H, CON*H*OH, *Z*-isomer), 10.78 (s, 0.6H, CON*H*OH, *E*-isomer); ^{13}C NMR (50 MHz, DMSO-d_6) δ 25.9 (*C*H_3), 39.8 (*C*H_2CONHOH), 44.1 (5-C), 62.6 (3-C), 123.9, 128.4, 144.6, 147.6 (aromatic C), 163.4 (*C*ONHOH, *E*-isomer), 168.7 (*C*ONHOH, *Z*-isomer), 168.0, 170.6 (2,6-C). Anal. Calcd for $C_{13}H_{15}ClN_4O_6$: C, 43.52; H, 4.21; N, 15.62; Found: C, 43.08; H, 4.46; N, 15.91.

4.1.48. N-Hydroxy-3,4-dimethyl-2,6-dioxo-3-phenyl-1-piperazineacetamide **11**

The *N*-benzyloxy precursor **61** was prepared from carboxylic acid **57** (680 mg, 2.46 mmol) following the procedure described for the preparation of compound **37** (precursor for **6**). The crude oil was purified by flash column chromatography eluting with AcOEt-*n*-hexane 1:1 to afford **61** as a clear glass oil (700 mg, 75%). This compound appears in the ^{1}H and ^{13}C NMR spectra as a mixture of *E*/*Z* conformers (not assigned). ^{1}H NMR (600 MHz, $CDCl_3$, 283 K) δ 1.65 (s , 3H, 3-CH_3), 2.57, 2.58 (s + s, 3H, 4-CH_3), 3.36-3.44 (dd, 1H, *J*=8.4, 20.4 Hz, 5-H), 3.65 (t, 1H, *J*=15.6, 16.8 Hz, 5-H), 4.30-4.43 (q, AB, 0.96H, J_{AB}=15.0 Hz, CH_2CONHOCH_2Ph), 4.75 (d, 1.1H, *J*=4.2 Hz, CH_2CONHOCH_2Ph), 4.89, 4.92 (s + s, 2H, CONHOCH_2Ph), 7.27-7.50 (complex m, 10H, aromatic H), 8.52 (s, 0.45H, CON*H*OCH_2Ph), 9.0 (s, 0.44H, CON*H*OCH_2Ph); ^{13}C NMR (150 MHz, $CDCl_3$, 283 K) δ 24.2 (3-*C*H_3), 38.1 (4-*C*H_3), 39.1, 39.5 (*C*H_2CONHOCH_2Ph), 54.9 (5-C), 67.8 (3-C), 78.1, 79.5 (CONHO*C*H_2Ph), 125.9, 126.0, 128.0, 128.1, 128.4, 128.5, 128.6, 128.8, 129.1, 129.3, 134.0, 135.1, 140.5 (aromatic C), 165.0, 170.2 (*C*ONHOCH_2Ph), 170.5, 173.7 (2,6-C); HRMS (ESI): $[M+H]^+$ calcd for $C_{21}H_{23}N_3O_4$, 382.1767, found 382.1753.

Compound **61** (720 mg, 1.89 mmol) was subjected to catalytic hydrogenation (H_2/10% Pd-C, 86 mg) in abs EtOH (85 mL) as described for the preparation of compound **6** from **37**. The crude hydrogenation product (off-white foamy solid) was chromatographed over flash silica eluting with AcOEt to afford the title compound **11** as a white foamy solid, which strongly binds the aforementioned solvent. Removal of the entrapped solvent as in **6** gave **11** as a slightly off-yellow solid (495 mg, 90%): mp 158-160 °C (dec); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.56 (s, 3H, 3-CH_3), 2.39, 2.41 (s + s, 3H, 4-CH_3), 3.31-3.59 (q, AB, 2H, J_{AB}=17.9 Hz, 5-H), 4.29 (t like, 1.47H, *J*=16.0 Hz, CH_2CONHOH, *E*-isomer), 4.59 (s, 0.46H, CH_2CONHOH, *Z*-isomer), 7.28-7.50 (complex m, 5H, aromatic H), 8.95 (s, 0.7H, CONHO*H*, *E*-isomer), 9.38 (s, 0.2H, CONHO*H*, *Z*-isomer), 10.27 (s, 0.2H, CON*H*OH, *Z*-isomer), 10.73 (s, 0.7H, CON*H*OH, *E*-isomer); ^{13}C NMR (100 MHz, DMSO-d_6) δ 22.4, 22.5 (3-*C*H_3), 37.3 (4-*C*H_3), 38.8 (*C*H_2CONHOH, *E*-isomer), 39.7 (*C*H_2CONHOH, *Z*-isomer), 54.0 (5-C), 67.3 (3-C), 126.5, 127.9, 128.5 (2,3,4,5,6-aromatic C), 140.7 (1-aromatic C), 163.8 (*C*ONHOH, *E*-isomer), 169.2 (*C*ONHOH, *Z*-isomer), 169.8, 169.9, 173.5 (2,6-C); HRMS (ESI): $[M+H]^+$ calcd for $C_{14}H_{17}N_3O_4$, 292.1297, found 292.1295. The hydrochloride salt (**11·HCl**) was prepared as described for **6·HCl**. Mp 153-156 °C (dec) (slightly hygroscopic). Anal. Calcd for $C_{14}H_{18}ClN_3O_4$: C, 51.30; H, 5.54; N, 12.82; Found: C, 50.94; H, 5.92; N, 12.51.

4.1.49. N-Hydroxy-4-methyl-2,6-dioxo-3-phenyl-3-propyl-1-piperazineacetamide **12**

The *N*-benzyloxy precursor **62** was prepared from carboxylic acid **58** (840 mg, 2.76 mmol) following the procedure described for the preparation of compound **37** (precursor for **6**). The crude viscous oil was purified by column chromatography on silica gel with AcOEt-*n*-hexane 2:3, as eluent, to afford **62** as a glass solid (790 mg, 69%). This compound appears in the ^{1}H and ^{13}C NMR spectra as a mixture of *E*/*Z* conformers (not assigned). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.73 (t, 3H, *J*=7.1 Hz, C*H*$_3$(CH$_2$)$_2$), 1.02-1.20 (m, 2H, CH$_3$C*H*$_2$CH$_2$), 1.76-2.10 (dm, 2H, CH$_3$CH$_2$C*H*$_2$), 2.44 (s, 3H, 4-C*H*$_3$), 3.23-3.70 (q, AB, 2H, J_{AB}=18.0 Hz, 5-H), 4.12-4.37 (~br s, 1H, C*H*$_2$CONHOCH$_2$Ph), 4.55, 4.61 (s + s, 0.9H, C*H*$_2$CONHOCH$_2$Ph), 4.78 (s, 2H, CONHOC*H*$_2$Ph), 7.08-7.40 (complex m, 10H, aromatic H), 8.67-8.86 (br s, 0.32H, CON*H*OCH$_2$Ph), 9.24-9.45 (br s, 0.41H, CON*H*OCH$_2$Ph); ^{13}C NMR (50 MHz, $CDCl_3$) δ 14.2 (*C*H$_3$(CH$_2$)$_2$), 16.2 (CH$_3$*C*H$_2$CH$_2$), 37.7 (4-*C*H$_3$), 38.8 (CH$_3$CH$_2$*C*H$_2$), 39.4 (*C*H$_2$CONHOCH$_2$Ph), 55.0 (5-C), 70.3 (3-C), 78.2, 79.5 (CONHO*C*H$_2$Ph), 127.0, 128.0, 128.5, 129.2, 134.4, 135.3, 136.9 (aromatic C), 165.2, 170.5, 173.7 (*C*ONHOCH$_2$Ph, 2,6-C); EI MS: m/z 410.3 ([M+H]$^+$, 2), 409.2 ([M]$^+$, 9), 367.2 ([M+H-CH$_2$CH$_2$CH$_3$]$^+$, 21), 366.2 ([M-CH$_2$CH$_2$CH$_3$]$^+$, 100), 338.2 (22), 231.2 (79), 174.1 (93), 91.0 (16).

Compound **62** (1.33 g, 3.25 mmol) was subjected to catalytic hydrogenation (H_2/10% Pd-C, 160 mg) in abs EtOH (146 mL) as described for the preparation of compound **6** from **37**. The crude hydrogenation product (off-white foamy solid) was chromatographed on silica gel column with AcOEt, as eluent, to afford the title compound **12** as a white foamy solid, which strongly binds the elution solvent. Removal of the entrapped solvent as in **6** gave **12** as a glass solid (903 mg, 87%): ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.81 (t, 3H, *J*=7.2 Hz, C*H*$_3$(CH$_2$)$_2$), 1.0-1.32 (dm, 2H, CH$_3$C*H*$_2$CH$_2$), 1.87 (t, 1H, *J*=10.8, 11.8 Hz, CH$_3$CH$_2$C*H*H), 2.07-2.23 (td, 1H, *J*=2.0, 4.2, 12.6, 13.6 Hz, CH$_3$CH$_2$CH*H*), 2.37, 2.38 (s + s, 3H, 4-C*H*$_3$), 3.26-3.57 (q, AB, 2H, J_{AB}=18.0 Hz, 5-H), 4.29 (s, 1.3H, C*H*$_2$CONHOH, *E*-isomer), 4.58 (s, 0.5H, C*H*$_2$CONHOH, *Z*-isomer), 7.27-7.47 (m, 5H, aromatic H), 8.97 (s, 0.6H, CONHO*H*, *E*-isomer), 9.40 (s, 0.2H, CONHO*H*, *Z*-isomer), 10.27 (s, 0.2H, CON*H*OH, *Z*-isomer), 10.74 (s, 0.6H, CON*H*OH, *E*-isomer); ^{13}C NMR (50 MHz, DMSO-d_6) δ 14.1 (*C*H$_3$(CH$_2$)$_2$), 16.4 (CH$_3$*C*H$_2$CH$_2$), 36.9 (4-*C*H$_3$), 37.7 (CH$_3$CH$_2$*C*H$_2$), 39.0 (*C*H$_2$CONHOH, *E*-isomer), 39.8 (*C*H$_2$CONHOH, *Z*-isomer), 53.9 (5-C), 70.0 (3-C), 127.2, 127.9, 128.3 (2,3,4,5,6-aromatic C), 136.2 (1-aromatic C), 163.8 (*C*ONHOH, *E*-isomer), 169.2 (*C*ONHOH, *Z*-isomer), 169.7, 173.3 (2,6-C); HRMS (ESI): [M+H]$^+$ calcd for $C_{16}H_{21}N_3O_4$, 320.1610, found 320.1617. The hydrochloride salt (**12·HCl**) was prepared by treating a diethyl ether solution of **12** with saturated solution of HCl in Et_2O under ice cooling. The resulting white solid was collected by vacuum filtration, triturated with Et_2O, and dried in vacuo (decomposed gradually above 110 °C). Anal. Calcd for $C_{16}H_{22}ClN_3O_4$: C, 54.01; H, 6.23; N, 11.81; Found: C, 53.65; H, 5.96; N, 11.62.

4.1.50. 3-Butyl-N-hydroxy-4-methyl-2,6-dioxo-3-phenyl-1-piperazineacetamide **13**

The *N*-benzyloxy precursor **63** was prepared from carboxylic acid **59** (1.2 g, 3.77 mmol) following the procedure described for the preparation of compound **37** (precursor for **6**). The crude yellowish oil was purified by flash column chromatography eluting with AcOEt-*n*-hexane 2:3 to afford **63** as a white foamy solid, which strongly binds the elution solvents. Removal of the entrapped solvents as in **37** gave **63** as a glass solid (1 g, 63%). This compound appears in the ^{1}H and ^{13}C NMR spectra as a mixture of *E*/*Z* conformers (not assigned). ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.74 (t, 3H, *J*=7.1 Hz, C*H*$_3$(CH$_2$)$_3$), 1.03-1.21 (m, 4H, CH$_3$C*H*$_2$C*H*$_2$CH$_2$), 1.84-2.10 (dm, 2H, CH$_3$(CH$_2$)$_2$C*H*$_2$), 2.46 (s, 3H, 4-C*H*$_3$), 3.26-3.68 (q, AB, 2H, J_{AB}=18.0 Hz, 5-H), 4.18-4.38 (br d, 1H, *J*=24.0 Hz, C*H*$_2$CONHOCH$_2$Ph), 4.53-4.69 (br s, 1H, C*H*$_2$CONHOCH$_2$Ph), 4.81 (s, 2H, CONHOC*H*$_2$Ph), 7.14-7.38 (complex m, 10H, aromatic H), 8.40-8.65 (br s, 0.6H, CON*H*OCH$_2$Ph), 8.85-9.10 (br s, 0.6H, CON*H*OCH$_2$Ph); ^{13}C NMR (50 MHz, $CDCl_3$) δ 14.0 (*C*H$_3$(CH$_2$)$_3$), 22.9 (CH$_3$*C*H$_2$(CH$_2$)$_2$), 24.9 (CH$_3$CH$_2$*C*H$_2$CH$_2$), 36.4 (CH$_3$(CH$_2$)$_2$*C*H$_2$), 37.8 (4-*C*H$_3$), 39.5 (*C*H$_2$CONHOCH$_2$Ph), 55.1 (5-C), 70.3 (3-C), 78.3, 79.5 (CONHO*C*H$_2$Ph), 127.1, 128.1, 128.6, 129.3, 134.4, 135.3, 136.8 (aromatic C), 165.2, 170.6, 173.8 (*C*ONHOCH$_2$Ph, 2,6-C); ESI$^+$ MS: m/z 424.4 [M+H]$^+$.

Compound **63** (900 mg, 2.13 mmol) was subjected to catalytic hydrogenation (H_2/10% Pd-C, 108 mg) in abs EtOH (96 mL) as described for the preparation of **6** from **37**. The crude hydrogenation product (off-white foamy solid) was chromatographed on silica gel column with AcOEt, as eluent, to afford the title compound **13** as a white

foamy solid, which strongly binds the elution solvent. Removal of the entrapped solvent as in **6** gave **13** as a glass solid (665 mg, 94%): ^{1}H NMR (600 MHz, DMSO-d_6) δ 0.81 (t, 3H, J=7.1 Hz, CH_3(CH$_2$)$_3$), 0.99-1.13 (m, 1H, CH$_3$CH$_2$CHHCH$_2$), 1.15-1.30 (m, 3H, CH$_3$CH_2CHHCH$_2$), 1.84-1.96 (m, 1H, CH$_3$(CH$_2$)$_2$CHH), 2.12-2.24 (m, 1H, CH$_3$(CH$_2$)$_2$CHH), 2.37, 2.38 (s + s, 3H, 4-CH_3), 3.29-3.56 (q, AB, 2H, J_{AB}=18.0 Hz, 5-H), 4.27 (s, 1.45H, CH_2CONHOH, *E*-isomer), 4.57 (s, 0.5H, CH_2CONHOH, *Z*-isomer), 7.30-7.45 (m, 5H, aromatic H), 8.92 (s, 0.6H, CONHOH, *E*-isomer), 9.34 (s, 0.2H, CONHOH, *Z*-isomer), 10.24 (s, 0.2H, CONHOH, *Z*-isomer), 10.70 (s, 0.6H, CONHOH, *E*-isomer); ^{13}C NMR (50 MHz, DMSO-d_6) δ 13.9 (CH_3(CH$_2$)$_3$), 22.4 (CH$_3$CH_2(CH$_2$)$_2$), 25.2 (CH$_3$CH$_2$CH_2CH$_2$), 35.2 (CH$_3$(CH$_2$)$_2$CH_2), 36.8 (4-CH_3), 39.0 (CH_2CONHOH, *E*-isomer), 39.8 (CH_2CONHOH, *Z*-isomer), 53.9 (5-C), 69.9 (3-C), 127.3, 128.0, 128.3 (2,3,4,5,6-aromatic C), 136.2 (1-aromatic C), 163.8 (C=ONHOH, *E*-isomer), 169.2 (C=ONHOH, *Z*-isomer), 169.7, 173.3 (2,6-C); HRMS (ESI): [M+H]$^+$ calcd for $C_{17}H_{23}N_3O_4$, 334.1767, found 334.1761. The hydrochloride salt (**13·HCl**) was prepared as described for **12·HCl**. Mp 162-166 °C (dec). Anal. Calcd for $C_{17}H_{24}ClN_3O_4$: C, 55.21; H, 6.54; N, 11.36; Found: C, 55.52; H, 6.23; N, 11.56.

4.1.51. 3-(4-Fluorophenyl)-N-hydroxy-3,4-dimethyl-2,6-dioxo-1-piperazineacetamide **14**

The *N*-benzyloxy precursor **64** was prepared from carboxylic acid **60** (1 g, 3.4 mmol) in dry THF-DMF 4:1 following the procedure described for the preparation of compound **40** (precursor for **9**). The crude oil was purified by column chromatography on silica gel eluting with AcOEt-*n*-hexane 1:2 and then 1:1 to afford **64** as a clear glass oil (1.03 g, 76%). This compound appears in the ^{1}H and ^{13}C NMR spectra as a mixture of *E*/*Z* conformers (not assigned). ^{1}H NMR (400 MHz, CDCl$_3$) δ 1.63 (s, 3H, 3-CH_3), 2.53 (s, 3H, 4-CH_3), 3.37-3.66 (q, AB, 2H, J_{AB}=18.0 Hz, 5-H), 4.26-4.50 (br d, 0.9H, J=21.2 Hz, CH_2CONHOCH$_2$Ph), 4.69-4.79 (br s, 1H, CH_2CONHOCH$_2$Ph), 4.91 (s, 2H, CONHOCH_2Ph), 7.04 (t, 2H, $J\simeq$8.7 Hz, 3,5-H for 4-FC$_6$H$_4$), 7.29-7.50 (complex m, 7H, C$_6H_5$, 2,6-H for 4-FC$_6$H$_4$), 8.31 (br s, 0.4H, CONHOCH$_2$Ph), 8.73 (br s, 0.4H, CONHOCH$_2$Ph); ^{13}C NMR (150 MHz, CDCl$_3$) δ 23.9 (3-CH_3), 38.2 (4-CH_3), 39.6 (CH_2CONHOCH$_2$Ph), 54.9 (5-C), 67.5 (3-C), 78.4, 79.7 (CONHOCH_2Ph), 115.7, 115.9 (d, $J_{C\text{-}F}$=21.2 Hz, 3,5-C for 4-FC$_6$H$_4$), 128.1, 128.2 (d, $J_{C\text{-}F}$=7.5 Hz, 2,6-C for 4-FC$_6$H$_4$), 128.5, 128.6, 128.8, 129.3, 129.4 (2,3,4,5,6-C for C$_6$H$_5$), 134.3, 135.2 (1-C for C$_6$H$_5$), 136.6 (1-C for 4-FC$_6$H$_4$), 161.7, 163.3 (d, $J_{C\text{-}F}$=246 Hz, 4-C for 4-FC$_6$H$_4$), 165.2 (weak signal intensity), 170.3, 173.7 (C=ONHOCH$_2$Ph, 2,6-C); ESI$^+$ MS: m/z 400.2 [M+H]$^+$.

Compound **64** (1 g, 2.5 mmol) was subjected to catalytic hydrogenation (H$_2$/10% Pd-C, 120 mg) in abs EtOH (112 mL) as described for the preparation of **6** from **37**. The crude hydrogenation product (off-white foamy solid) was chromatographed on silica gel column with AcOEt, as eluent, to afford the title compound **14** as a white foamy solid, which strongly binds the elution solvent. Removal of the entrapped solvent as in **6** gave **14** as a glass solid (703 mg, 91%): ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.56 (s, 3H, 3-CH_3), 2.35, 2.37 (s + s, 3H, 4-CH_3), 3.33-3.60 (q, AB, 2H, $J_{AB}\simeq$18.0 Hz, 5-H), 4.27 (s, 1.4H, CH_2CONHOH, *E*-isomer), 4.57 (s, 0.5H, CH_2CONHOH, *Z*-isomer), 7.19 (t, 2H, J=8.7 Hz, 3,5-aromatic H), 7.46-7.54 (~q, 2H, J=4.4, 5.4 Hz, 2,6-aromatic H), 8.96 (s, 0.7H, CONHOH, *E*-isomer), 9.39 (s, 0.2H, CONHOH, *Z*-isomer), 10.28 (s, 0.2H, CONHOH, *Z*-isomer), 10.74 (s, 0.6H, CONHOH, *E*-isomer); ^{13}C NMR (50 MHz, DMSO-d_6) δ 21.7, 21.8 (3-CH_3), 37.2 (4-CH_3), 38.9 (CH_2CONHOH, *E*-isomer), 39.7 (CH_2CONHOH, *Z*-isomer), 53.8 (5-C), 66.8 (3-C), 115.0, 115.5 (d, $J_{C\text{-}F}$=21.1 Hz, 3,5-aromatic C), 128.7, 128.9 (d, $J_{C\text{-}F}$=7.9 Hz, 2,6-aromatic C), 136.9, 137.0 (d, $J_{C\text{-}F}$=3.1 Hz, 1-aromatic C), 159.2, 164.0 (d, $J_{C\text{-}F}$=243 Hz, 4-aromatic C), 163.8 (C=ONHOH, *E*-isomer), 169.2 (C=ONHOH, *Z*-isomer), 169.6, 173.4 (2,6-C); HRMS (ESI): [M+Na]$^+$ calcd for $C_{14}H_{16}FN_3O_4$, 332.1023, found 332.1021. The hydrochloride salt (**14·HCl**) was prepared as described for **12·HCl** (decomposed gradually above 126 °C). Anal. Calcd for $C_{14}H_{17}ClFN_3O_4$: C, 48.63; H, 4.96; N, 12.15; Found: C, 48.34; H, 5.27; N, 11.94.

4.1.52. N-Hydroxy-3,4-dimethyl-3-(4-nitrophenyl)-2,6-dioxo-1-piperazineacetamide **15**

The *N*-(4-methoxybenzyloxy) precursor **65** was prepared from 4-methoxybenzyl ester **56** (1 g, 2.27 mmol) by the same method as for **41** (precursor for **10**). The crude oil was purified by column chromatography on silica gel with AcOEt-*n*-hexane 1:1, as eluent, to afford **65** as a yellowish thick oil (445 mg, 43% yield over two steps). This compound appears in the ^{1}H NMR spectrum as two pairs of rotamers for the *E*/*Z* isomers as deduced both from the number of signals ad their integration. ^{1}H NMR (400 MHz, CDCl$_3$) δ [1.60 (s), 1.66 (s)] 3H, 3-CH_3, [2.21 (s),

2.54 (s)] 3H, 4-C*H*$_3$, [2.82-3.03 (q, *J*=13.4, 16.3 Hz), 3.43-3.62 (q, AB, J_{AB}=18.1 Hz)] 2H, 5-H, [3.77 (s), 3.80 (s)] OC*H*$_3$, 4.26-4.47 (q, *J*=14.0, 16.7 Hz, C*H*$_2$CONHOCH$_2$C$_6$H$_4$OCH$_3$-4), 4.60 (s, CONHOC*H*$_2$C$_6$H$_4$OCH$_3$-4), [4.69 (s), 4.71 (s)] C*H*$_2$CONHOC*H*$_2$C$_6$H$_4$OCH$_3$-4, 4.83 (s, CONHOC*H*$_2$C$_6$H$_4$OCH$_3$-4), [6.83 (d, *J*=8.3 Hz), 6.89 (d, *J*=8.1 Hz)] 3,5-H for 4-CH$_3$OC$_6$H$_4$), [7.23 (d, *J*=8.1 Hz), 7.26-7.37 (q, *J*=8.4, 9.0 Hz)] 2,6-H for 4-CH$_3$OC$_6$H$_4$), [7.63 (d, *J*=7.6 Hz), 7.70 (d, *J*=8.6 Hz)] 2,6-H for 4-NO$_2$C$_6$H$_4$), [8.05-8.17 (m), 8.20 (d, *J*=8.5 Hz)] 3,5-H for 4-NO$_2$C$_6$H$_4$), [8.61 (br s), 9.09 (br s), 10.11 (br s), 10.38 (br s)] CON*H*OCH$_2$C$_6$H$_4$OCH$_3$-4); ^{13}C NMR (50 MHz, CDCl$_3$) δ 23.3 (3-*C*H$_3$), 37.8 (weak signal intensity), 38.2 (4-*C*H$_3$), 39.6 (*C*H$_2$CONHOCH$_2$C$_6$H$_4$OCH$_3$-4), 54.7 (5-C), 55.3 (O*C*H$_3$), 67.8 (3-C), 77.7, 79.4 (CONHO*C*H$_2$C$_6$H$_4$OCH$_3$-4), 113.9, 114.1, 123.6, 124.1, 127.6, 128.2, 129.3, 130.2, 131.0, 131.1, 147.2, 147.7, 148.3 (aromatic C), 159.6, 160.1 (*C*ONHOCH$_2$C$_6$H$_4$OCH$_3$-4), 169.6, 172.7 (2,6-C); ESI$^+$ MS: m/z 457.2 [M+H]$^+$.

Compound **65** (460 mg, 1.01 mmol) was subjected to treatment with TFA (7.4 mL) and Et$_3$SiH (742 µL) in dry CH$_2$Cl$_2$ (28 mL) as described for the preparation of compound **10** from **41**. The crude product (yellow foamy solid) was chromatographed on silica gel column eluting successively with AcOEt-*n*-hexane 2:1, AcOEt and AcOEt-MeOH 9:1 to afford the title compound **15** as a pale yellow foamy solid, which strongly binds the aforementioned solvents. Removal of the entrapped solvents as in **6** gave **15** as a light-yellow solid (237 mg, 70%): ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 1.61 (s, 3H, 3-C*H*$_3$), 2.36, 2.39 (s + s, 3H, 4-C*H*$_3$), 3.41-3.66 (q, AB, 2H, J_{AB}=18.0 Hz, 5-H), 4.27 (s, 1.4H, C*H*$_2$CONHOH, *E*-isomer), 4.51-4.65 (q, AB, 0.6H, J_{AB}=17.0 Hz, C*H*$_2$CONHOH, *Z*-isomer), 7.76 (d, 2H, *J*=8.8 Hz, 2,6-aromatic H), 8.22 (d, 2H, *J*=8.8 Hz, 3,5-aromatic H), 8.96 (s, 0.7H, CONHO*H*, *E*-isomer), 9.40 (s, 0.3H, CONHO*H*, *Z*-isomer), 10.30 (s, 0.2H, CON*H*OH, *Z*-isomer), 10.74 (s, 0.7H, CON*H*OH, *E*-isomer); ^{13}C NMR (50 MHz, DMSO-*d*$_6$) δ 20.9 (3-*C*H$_3$), 37.2 (4-*C*H$_3$), 38.9 (*C*H$_2$CONHOH), 53.7 (5-C), 67.2 (3-C), 123.6 (3,5-aromatic C), 128.2 (2,6-aromatic C), 147.1, 148.6 (1,4-aromatic C), 163.6 (*C*ONHOH, *E*-isomer), 169.0 (*C*ONHOH, *Z*-isomer), 169.3, 172.8 (2,6-C); HRMS (ESI): [M+H]$^+$ calcd for C$_{14}$H$_{16}$N$_4$O$_6$, 337.1148, found 337.1157.

The hydrochloride salt (**15·HCl**) was prepared by the same way described for **10**, and obtained as an off-white slightly hygroscopic solid (decomposed gradually above 166 °C). Anal. Calcd for C$_{14}$H$_{17}$ClN$_4$O$_6$: C, 45.11; H, 4.60; N, 15.03; Found: C, 44.78; H, 4.52; N, 15.28.

4.1.53. N-Hydroxy-3-methyl-2,6-dioxo-3-phenyl-4-propyl-1-piperazineacetamide Hydrochloride **16**

The *N*-benzyloxy precursor **74** was prepared from carboxylic acid **72** (496 mg, 1.63 mmol) following the procedure described for the preparation of compound **37** (precursor for **6**). The crude yellowish thick oil was purified by column chromatography on silica gel with AcOEt-*n*-hexane 2:3, as eluent, to afford **74** as a glass solid (433 mg, 65%). This compound appears in the ^{1}H and ^{13}C NMR spectra as a mixture of *E*/*Z* conformers (not assigned). ^{1}H NMR (400 MHz, CDCl$_3$) δ 0.92 (t, 3H, *J*=7.4 Hz, NCH$_2$CH$_2$C*H*$_3$), 1.40-1.61 (m, 2H, NCH$_2$C*H*$_2$CH$_3$), 1.66 (s, 3H, 3-C*H*$_3$), 2.53-2.64 (br s, 1H, NC*H*HCH$_2$CH$_3$), 2.66-2.78 (m, 1H, NCH*H*CH$_2$CH$_3$), 3.45-3.70 (q, AB, 2H, J_{AB}=18.4 Hz, 5-H), 4.21-4.55 (v br d, 0.9H, C*H*$_2$CONHOCH$_2$Ph), 4.66-4.82 (br s, 0.9H, C*H*$_2$CONHOCH$_2$Ph), 4.91 (s, 2H, CONHOC*H*$_2$Ph), 7.22-7.53 (complex m, 10H, aromatic H), 7.99-8.40 (v br s, 0.4H, CON*H*OCH$_2$Ph), 8.42-8.85 (v br s, 0.3H, CON*H*OCH$_2$Ph); ^{13}C NMR (150 MHz, CDCl$_3$) δ 11.7 (NCH$_2$CH$_2$*C*H$_3$), 21.4 (NCH$_2$*C*H$_2$CH$_3$), 23.3 (3-*C*H$_3$), 39.8 (*C*H$_2$CONHOCH$_2$Ph), 50.7 (N*C*H$_2$CH$_2$CH$_3$), 51.1 (5-C), 68.2 (3-C), 78.4, 79.7 (CONHO*C*H$_2$Ph), 126.4, 128.3, 128.9, 129.4, 134.3, 135.3, 141.4 (aromatic C), 165.1, 170.3 (*C*ONHOCH$_2$Ph), 170.7, 174.0 (2,6-C); ESI$^+$ MS: m/z 410.4 [M+H]$^+$.

Compound **74** (390 mg, 0.95 mmol) was subjected to catalytic hydrogenation (H$_2$/10% Pd-C, 47 mg), in abs EtOH (43 mL) as described for the preparation of **6** from **37**. The crude hydrogenation material (off-white foamy solid) was chromatographed on silica gel column eluting first with AcOEt-*n*-hexane 2:1 and then AcOEt to afford the free base **16** as a white foamy solid, which strongly binds the elution solvents. (EI MS: m/z 319.1 ([M]$^+$, 13), 291.1 (14), 276.1 ([M-CH$_2$CH$_2$CH$_3$]$^+$, 11), 231.1 (26), 174.0 (100)). This free base product was converted into the HCl salt as described for compound **12** to give 238 mg (70%) of the title compound **16** as a white slightly hygroscopic solid (decomposed gradually above 115 °C); ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 0.78 (t, 3H, *J*=7.2 Hz, $^+$NH(CH$_2$)$_2$C*H*$_3$), 1.32-1.59 (complex m, 2H, $^+$NHCH$_2$C*H*$_2$CH$_3$), 1.73 (s, 3H, 3-C*H*$_3$), 2.34-2.53 (m, 1H, $^+$NHC*H*HCH$_2$CH$_3$), 2.62-2.88 (br s, 1H, $^+$NHCH*H*CH$_2$CH$_3$), 3.46-3.93 (q, AB, 2H, $J_{AB}\simeq$16.0 Hz, 5-H), 4.28 (s, 1.5H, C*H*$_2$CONHOH, *E*-isomer), 4.56 (s,

0.5H, CH_2CONHOH, *Z*-isomer), 6.52-8.41 (br m, 7H, CONHO*H*, $^+$N*H*$(CH_2)_2CH_3$, aromatic H), 10.29 (s, 0.2H, CON*H*OH, *Z*-isomer), 10.84 (s, 0.5H, CON*H*OH, *E*-isomer); ^{13}C NMR (50 MHz, DMSO-d_6) δ 11.3 ($^+$NH$(CH_2)_2$*C*H_3), 19.8 ($^+$NHCH_2*C*H_2CH$_3$), 20.6 (3-*C*H_3), 39.5 (*C*H_2CONHOH, *E*-isomer), 40.4 (*C*H_2CONHOH, *Z*-isomer), 50.0 (5-C), 50.6 ($^+$NH*C*H_2CH$_2$CH$_3$), 68.0 (3-C), 127.3, 128.6 (2,3,4,5,6-aromatic C), 139.1 (1-aromatic C), 163.5 (*C*ONHOH, *E*-isomer), 168.9 (*C*ONHOH, *Z*-isomer), 168.4, 172.6 (2,6-C). Anal. Calcd for $C_{16}H_{22}ClN_3O_4$: C, 54.01; H, 6.23; N, 11.81; Found: C, 53.66; H, 6.62; N, 12.14.

Note. The above prepared free base **16** was partially decomposed on attempted drying at 62-64 °C under high vacuum (10^{-2} mmHg).

4.1.54. 4-Butyl-N-hydroxy-3-methyl-2,6-dioxo-3-phenyl-1-piperazineacetamide Hydrochloride **17**

The *N*-benzyloxy precursor **75** was prepared from carboxylic acid **73** (465 mg, 1.46 mmol) following the procedure described for the preparation of compound **37** (precursor for **6**). The crude yellowish thick oil was purified by column chromatography on silica gel with AcOEt-*n*-hexane 1:2, as eluent, to afford **75** as a glass oil, which solidified under cooling (white solid, 400 mg, 65% yield): mp 128-129 °C (AcOEt/Et_2O 1:10-*n*-pentane). This compound appears in the ^{1}H and ^{13}C NMR spectra as a mixture of *E*/*Z* conformers (not assigned). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.90 (t, 3H, *J*=7.3 Hz, N$(CH_2)_3$CH_3), 1.24-1.55 (complex m, 4H, NCH_2CH_2CH_2CH$_3$), 1.49 (s, 3H, 3-CH_3), 2.50-2.67 (br s, 1H, NC*H*H$(CH_2)_2CH_3$), 2.69-2.82 (m, 1H, NCH*H*$(CH_2)_2CH_3$), 3.49-3.65 (q, AB, 2H, J_{AB}=18.2 Hz, 5-H), 4.15-4.53 (v br d, 0.8H, CH_2CONHOCH_2Ph), 4.72 (s, 0.9H, CH_2CONHOCH_2Ph), 4.89 (s, 2H, CONHOCH_2Ph), 7.20-7.53 (complex m, 10H, aromatic H), 8.30-8.47 (br s, 0.4H, CON*H*OCH_2Ph), 8.67-8.91 (br s, 0.4H, CON*H*OCH_2Ph); ^{13}C NMR (150 MHz, $CDCl_3$) δ 14.0 (N$(CH_2)_3$CH_3), 20.2 (N$(CH_2)_2$CH_2CH$_3$), 23.2 (3-CH_3), 30.4 (NCH_2CH_2CH_2CH$_3$), 39.7 (*C*H_2CONHOCH_2Ph), 48.4 (N*C*$H_2(CH_2)_2CH_3$), 51.0 (5-C), 68.1 (3-C), 78.3, 79.7 (CONHO*C*H_2Ph), 126.3, 128.1, 128.9, 129.4, 134.4, 135.3, 141.5 (aromatic C), 165.2, 170.3 (*C*ONHOCH_2Ph) 170.9, 174.1 (2,6-C). Anal. Calcd for $C_{24}H_{29}N_3O_4$: C, 68.06; H, 6.90; N, 9.92; Found: C, 68.32; H, 7.02; N, 9.63.

Compound **75** (620 mg, 1.46 mmol) was subjected to catalytic hydrogenation (H_2/10% Pd-C, 74 mg) in abs EtOH (66 mL) as described for the preparation of **6** from **37**. The crude hydrogenation material (off-white foamy solid) was chromatographed on silica gel column with AcOEt-*n*-hexane 1:1, as eluent, to afford the free base **17** as a white foamy solid, which strongly binds the elution solvents. (CI^+ MS: m/z 334.1 ($[M+H]^+$, 7), 333.1 ($[M]^+$, 6), 305.1 (17), 276.0 ($[M-CH_2CH_2CH_2CH_3]^+$, 10), 245.1 (35), 188.1 (100)). This free base product was converted into the HCl salt as described for compound **12** to give 369 mg (68%) of the title compound **17** as a white slightly hygroscopic solid (decomposed gradually above 108 °C); ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.67 (t, 3H, *J*=7.4 Hz, $^+$NH$(CH_2)_3$CH_3), 1.0-1.20 (complex m, 2H, $^+$NH$(CH_2)_2$CH_2CH$_3$), 1.22-1.36 (complex m, 2H, $^+$NHCH_2CH_2CH$_2$CH$_3$), 1.54 (s, 3H, 3-CH_3), 2.31-2.44 (m, 2H, $^+$NHC$H_2(CH_2)_2CH_3$), 3.30-3.68 (q, AB, 2H, J_{AB}=17.4 Hz, 5-H), 4.14 (s, 1.5H, CH_2CONHOH, *E*-isomer), 4.43 (s, 0.5H, CH_2CONHOH, *Z*-isomer), 5.40-6.80 (v br s, 2H, CONHO*H*, $^+$N*H*$(CH_2)_3CH_3$), 7.15-7.48 (m, 5H, aromatic H), 10.14 (s, 0.2H, CON*H*OH, *Z*-isomer), 10.66 (s, 0.5H, CON*H*OH, *E*-isomer); ^{13}C NMR (50 MHz, DMSO-d_6) δ 13.6 ($^+$NH$(CH_2)_3$CH_3), 19.5 ($^+$NH$(CH_2)_2$CH_2CH$_3$), 20.7 (3-CH_3), 29.0 ($^+$NHCH_2CH_2CH$_2$CH$_3$), 39.1 (CH_2CONHOH, *E*-isomer), 39.9 (CH_2CONHOH, *Z*-isomer), 48.0 ($^+$NHC$H_2(CH_2)_2CH_3$), 50.1 (5-C), 67.7 (3-C), 126.9, 128.1, 128.4 (2,3,4,5,6-aromatic C), 140.4 (1-aromatic C), 163.6 (*C*ONHOH, *E*-isomer), 169.0 (*C*ONHOH, *Z*-isomer), 169.1, 173.2 (2,6-C). Anal. Calcd for $C_{17}H_{24}ClN_3O_4$: C, 55.20; H, 6.54; N, 11.36; Found: C, 54.83; H, 6.86; N, 11.02.

Note. The above prepared free base **17** was partially decomposed on attempted drying at 62-64 °C under high vacuum (10^{-2} mmHg).

4.2 Biological Assays

4.2.1. Trypanocidal assays

Bloodstream form *T. brucei* (strain 221) were cultured in modified Iscove's medium, as outlined previously [11]. Eight-point potency curves were performed in 96 well plates (200 μL volumes), and the compound concentrations that inhibited growth by 50% (IC50) and 90% (IC90) were determined. Parasites were first diluted to 2.5×10^4 mL^{-1}, compounds were added at range of concentrations, and the plates incubated at 37 °C.

Resazurin was added after 48 h, and the plates incubated for a further 16 h. Fluorescence intensities were determined using a BMG FLUOstar Omega (excitation 545 nm, emission 590 nm). Data were analysed using Graph Pad Prism 7 software. Values are expressed as IC50 ± SD and are the average of three independent replicates.

4.2.2 *In vitro* cytotoxicity assays on rat skeletal myoblast L6 cells

Cytotoxicity against L6 cells was assessed using microtitre plates. Briefly, cells were seeded in triplicate at 1×10^4 mL^{-1} in growth medium containing different compound concentrations. The plates were incubated for 6 days at 37 °C and resazurin then added to each well. After a further 8 h incubation, the fluorescence was determined using a Spectramax plate reader.

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