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Affinity of 3-acyl substituted 4-quinolones at the benzodiazepine site of $GABA_A$ receptors

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ABSTRACT

The finding that alkyl 1,4-dihydro-4-oxoquinoline-3-carboxylate and *N*-alkyl-1,4-dihydro-4-oxoquinoline-3-carboxamide derivatives may be high-affinity ligands at the benzodiazepine binding site of the $GABA_A$ receptor, prompted a study of 3-acyl-1,4-dihydro-4-oxoquinoline (3-acyl-4-quinolones). In general, the affinity of the 3-acyl derivatives was found to be comparable with the 3-carboxylate and the 3-carboxamide derivatives, and certain substituents (e.g., benzyl) in position 6 were again shown to be important. As it is believed that the benzodiazepine binding site is situated between an α- and a γ-subunit in the $GABA_A$ receptor, selected compounds were tested on the $\alpha_1\beta_2\gamma_{2s}$, $\alpha_2\beta_2\gamma_{2s}$ and $\alpha_3\beta_2\gamma_{2s}$ $GABA_A$ receptor subtypes. The 3-acyl-4-quinolones display various degrees of selectivity for α_1- versus α_2- and α_3-containing receptors, and high-affinity ligands essentially selective for α_1 over α_3 were developed.

1. Introduction

γ-Aminobutyric acid, GABA, is a major neurotransmitter in the mammalian brain.[1] GABA binds to three different receptor types: the ligand gated chloride ion channels, $GABA_A$ and $GABA_C$ as well as the G-protein coupled receptor, $GABA_B$.[2] $GABA_A$ receptors are pentameric transmembrane proteins assembled by various combinations of seven different subunits, which are mostly available as multiple variants (α_{1-6}, β_{1-4}, γ_{1-4}, δ, ε, θ, and ρ_{1-3}).[3] The most common composition is two α, two β and one γ subunit. It is believed that receptors with different subtype compositions are responsible for different physiologic responses. Many ligands, such as benzodiazepines, barbiturates, ethanol and certain steroids, are known to allosterically alter the effect of GABA on the $GABA_A$ receptors.[1] The pharmacological properties of benzodiazepines (anxiolytic, anticonvulsant, muscle relaxant and sedative-hypnotic) have made them the most common $GABA_A$ receptors modulating drugs in clinical use,[4] and the benzodiazepine binding site is consequently an interesting target for development of novel drugs. Several other types of compound are known to bind to the benzodiazepine binding site, such as the pyrimidin-5(6H)-ones, the β-carbolines, the triazolopyridazines, the pyrazolo-quinolinones, the cyclopyrrolones, the pyridodiindoles and the quinolines.[5–7] The benzodiazepine binding site is believed to be situated in the interface between an α- and a γ-subunit, and a pharmacophore model of the binding site has been developed.[8] We have developed and refined this model with additional pharmacophore elements following a SAR study based on synthetic flavone derivatives.[9,10] The model has successfully guided us in identifying and optimizing novel 4-quinolone derivatives of types A and B (see Fig. 1).[11,12] Not only was it possible to prepare very potent 4-quinolones, showing K_i values down to 0.05 nM, but a preliminary study also indicated that some of these compounds show significant subtype selectivity.[12] However, 4-quinolones of types A and B can be expected to be hydrolyzed rapidly by ester/amide hydrolysis in vivo, and are mainly useful for the development of the pharmacophore model. In the present study, we have therefore focused on three issues:

- To investigate the effect of an acyl substituent in position 3, instead of a carboxylate or carboxamide, by preparing metabolically more stable ketone analogues (i.e., type C) of the esters and amides in Figure 1.
- To further explore the pharmacophore region called 'interface' (see Fig. 2) that has been proposed to be a channel-like cavity at the interface between an α- and a γ-subunit.[10]
- To further evaluate the subtype selectivity of 4-quinolones.

Differences between subtypes has been evaluated as it is believed that different subtypes mediate different physiological

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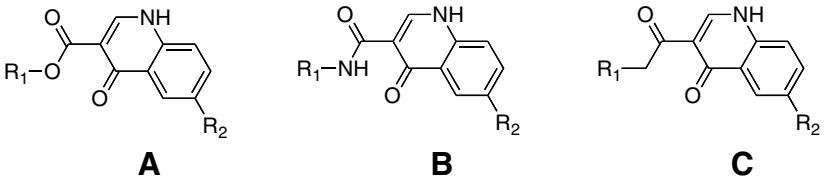


Figure 1. 4-Quinolone derivatives with affinity for the benzodiazepine site.

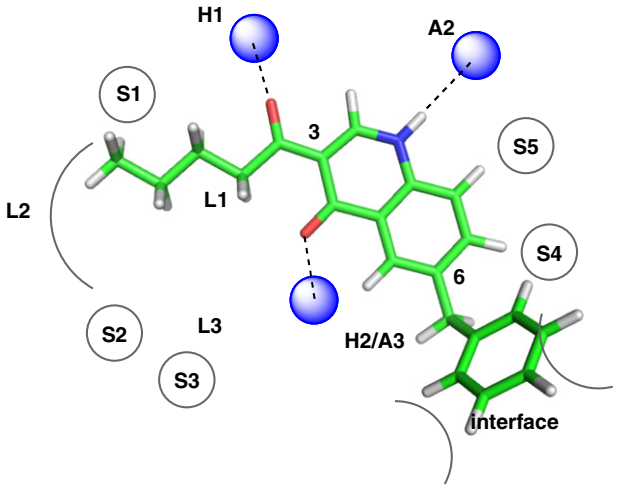


Figure 2. The proposed binding mode of **14** in the pharmacophore model of the benzodiazepine site of the $GABA_A$ receptors. The conformation of the 6-benzyl group is arbitrary.

effects: α_1-containing receptors are involved with anterograde amnesia and sedation, whereas α_2-, α_3- and possibly α_5-containing receptors are involved with anxiolytic activity.[13,14] Consequently, a subtype-selective compound could discriminate between the many behaviors mediated by GABA transmission.

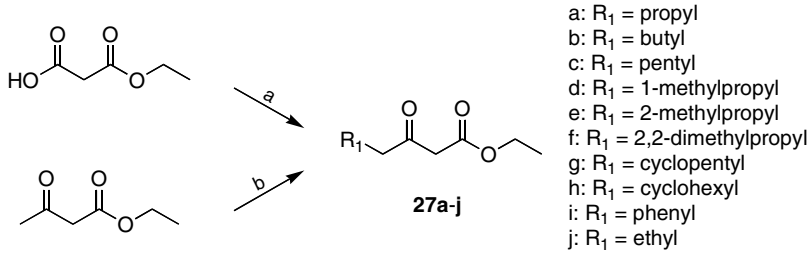
2. Chemistry

The 24 3-acyl-4-quinolone derivatives prepared and investigated in this study are to our knowledge all new compounds. Important building blocks for the synthesis of the 4-quinolones are the β-keto esters **27a**–**j**, which were prepared by two different routes outlined in Scheme 1 or purchased (**27j**).[15,16] The β-keto esters **27a**–**j** were converted to the corresponding 2-acyl-3-ethoxy acrylate derivatives, by condensation with triethyl orthoformate in the presence of acetic anhydride (see Scheme 3), which were used in the next step without purification or characterization. The different substituents in the 6-position were introduced by utilizing the corresponding aniline derivatives, synthesized according to Scheme 2 if not commercially available (aniline, *p*-ethylaniline, *p*-phenylaniline, *p*-benzylaniline, *p*-bromoaniline). The nitro precursors **28**–**30** were synthesized by various routes, including a Heck coupling,[17] a Wittig reaction[18] and a Suzuki coupling.[19] The nitrobenzyl-pyridine derivative **31** was purchased, **33** was obtained by reduction of the keto functionality of 3-benzoylpyridine[20] to yield **32**, followed by nitration,[21] while **34** was prepared by nitration of 2-benzylpyridine.[22] The nitro compounds were reduced to the corresponding anilines **35**–**40** with hydrogen in the presence of palladium on charcoal. A set of 3-acyl-4-quinolones were synthesized by reacting the 2-acyl-3-ethoxy acrylate derivatives (see Scheme 3) with the anilines, to give the enamines **41**–**62**.[23,24] The enamines were isolated as *Z*/*E* mixtures, although the *E* isomer was the major component (>90%) in all cases as indicated by NMR. The enamines were subsequently cyclized in refluxing diphenyl ether, yielding the desired 3-acyl-4-quinolone derivatives **1**–**10** and **12**–**23** (see Table 1 and Section 5 for definition of R_1 and R_2 in each compound). Compound **11** was obtained by direct nitration of **10**.[25] The 5,6,7,8-tetrahydro-4-quinolone **24** was prepared by the procedure depicted in Scheme 4[26]. The condensation of the β-keto ester **27a** with triethyl orthoformate in the presence of acetic anhydride followed by the treatment of the resulting intermediate with ammonia, resulted in the formation of amino analog **63** which was converted to **64** by the condensation with cyclohexanone. **24** was obtained by cyclization of **64** in refluxing diphenyl ether.

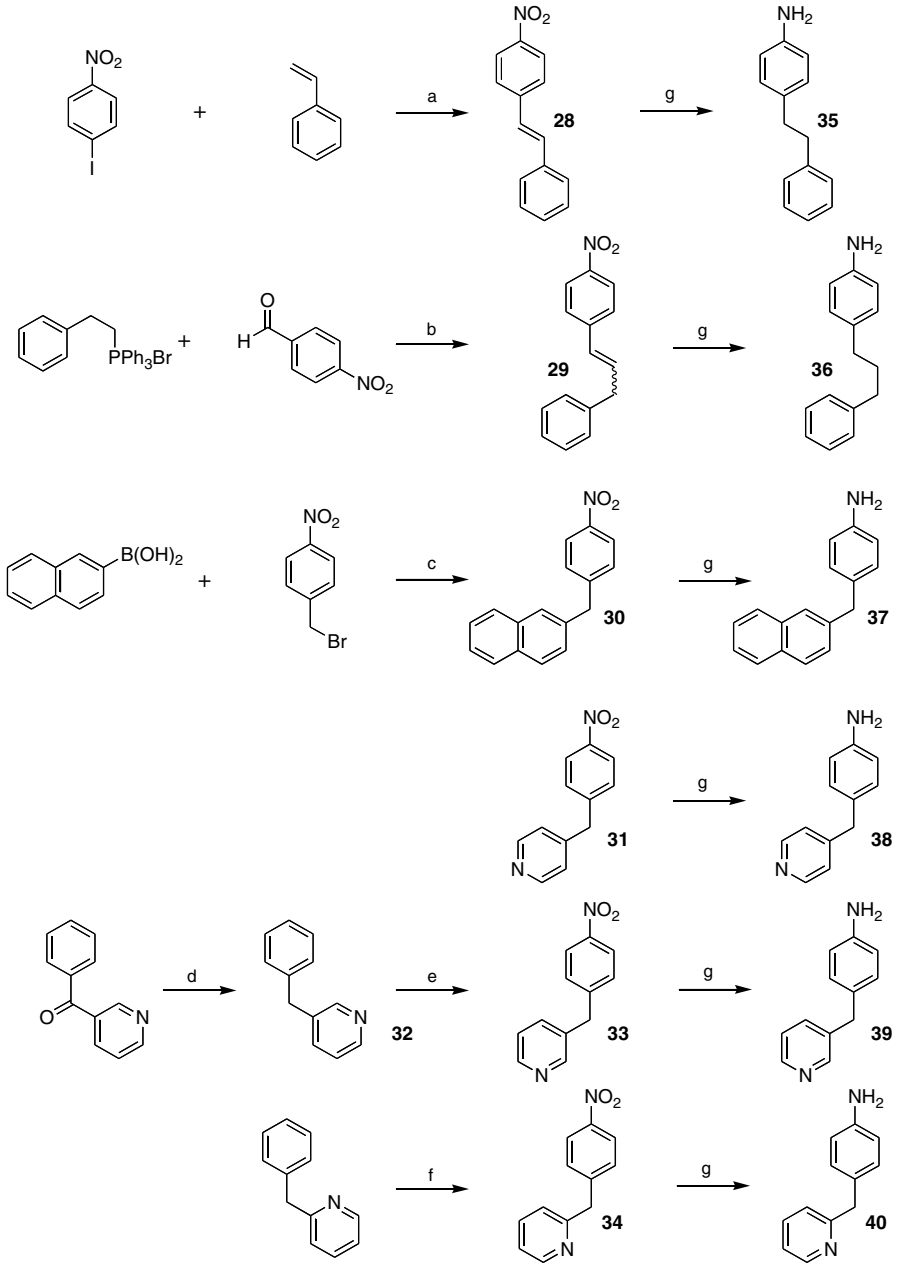
3. Results and discussion

The affinities of the tested 3-acyl-4-quinolone derivatives at the benzodiazepine binding site were determined by displacement of ^{3}H-flumazenil in rat cortical tissue in vitro (see the Section 5 for details). The results are presented in Table 1. As discussed in detail below, the affinities of the 3-acyl-4-quinolones are comparable with those of the corresponding 3-carboxylates and 3-carboxamides, indicating that the proposed interaction of a keto carbonyl group with the hydrogen bond donor (H1, see Fig. 2) is comparable with that of the ester or amide.

Our previous investigation of the corresponding 3-carboxylates and 3-carboxamides has indicated that a chain length of the 3-substituent of 5 (including the ester/amide functionality) is optimal,[12] and the focus of this investigation was therefore on the 3-valeryl derivatives. However, the length of the unbranched chain is not that critical in the acyl series, as discussed below. Branched or cyc-

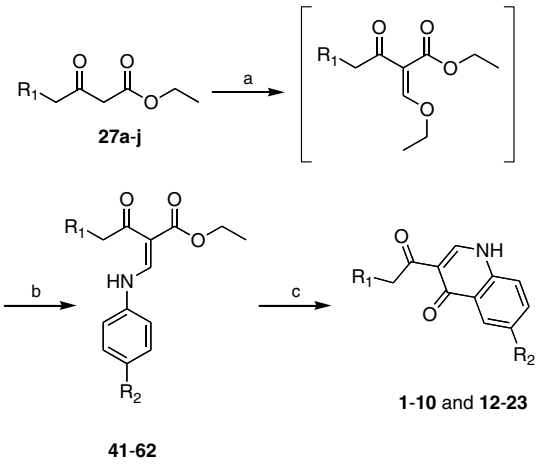


Scheme 1. Reagents and conditions: (a) 1—2 equiv *n*-BuLi, −78 to −5 °C, 10 min; 2—R_1CH_2COCl, −78 °C–rt, 15 min; (b) 1—NaH, 0 °C; 2—*n*-BuLi, 0 °C; 3—R_1-X, 0 °C–rt, 30 min.



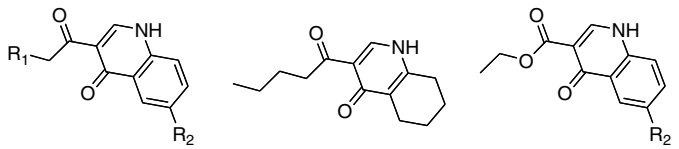
Scheme 2. Reagents and conditions: (a) K_2CO_3, tetrabutylammonium bromide, $Pd(OAc)_2$, DMF, 100 °C, 12 h; (b) *n*-BuLi, THF, −78 °C–rt; (c) $PdCl_2$, K_2CO_3, acetone/H_2O (3:1), 0 °C–rt, 3 h; (d) 10% Pd/C, concd HCl, H_2, rt, 24 h; (e) NH_4NO_3, $(CF_3CO)_2O$, $CHCl_3$, −10 °C–rt, 43 h; (f) concd HNO_3, concd H_2SO_4, 0–50 °C, 5 min; (g) H_2 (500 psi or 1 atm), 10% Pd/C, MeOH or EtOH or EtOH/EtOAC, rt, 3 h or over night.

lic groups in the ketone moiety (**4**–**9**) will result in higher K_i values than corresponding straight chain derivatives, as previously was shown for the esters.[12] The comparison of **25** and **26**, or the corresponding propyl esters and amides with R_2 either as an ethyl or as a benzyl group, shows that a benzyl group instead of an ethyl group in the 6-position increase the affinity by a factor 10–20.[12] Consequently, the receptor–ligand interaction in the vicinity of this position is interesting to explore further, keeping the rest of the molecule as the 3-valeryl-4-quinolone. By exchanging the ethyl group of **1** and **7** for a benzyl group (to **14** and **16**) the same effect can be seen for the 3-acyl-4-quinolones as for **25** and **26**, although less pronounced. A 6-phenyl (**13**) is obviously not good, resulting in a 165-fold decrease in affinity compared to the benzyl derivative **14**. The low affinity of **13** could be explained by the requirement for ligands to be planar in this receptor region.[9] Conformational analysis using the MMFF94s force field and the GB/SA hydration model, performed as described in Section 5, shows that the energy cost for the phenyl group of **13** to adopt a co-planar conformation with respect to the bicyclic system is 2.9 kcal compared to its global minimum. Increasing the linker length between position 6 and the benzene ring of **14** with one methylene group (**17**) has a small effect. However, with a propylene linker (**18**) or a larger aromatic group (**19**) the K_i values increase, indicating a steric repulsive interaction further out in the 'interface' and/or higher energy penalties due to the longer linker of **18** when adopting the right bioactive conformation. In spite of the significantly higher desolvation energy of pyridylmethylene compared to benzyl, compounds **20**–**22** display only a slightly lower affinity than corresponding benzyl compound **14**. An explanation may be that part of the 6-substituent is solvated, with the result that no extra desolvation energy



Scheme 3. Reagents and conditions: (a) triethyl orthoformate, acetic anhydride, 100 °C, over night; (b) *para*-substituted aniline derivatives, 100 °C, 1 h; (c) diphenyl ether, reflux, 30 min R_1 according to Scheme 1 and Table 1, R_2 according to Table 1.

Table 1
K_i values of 3-acyl-4-quinolones **1**–**23** and 3-pentanoyl-5,6,7,8-tetrahydro-4-quinolone **24** tested on ^{3}H-flumazenil binding in vitro to rat cortical membranes



Compound	R_1	R_2	K_i value [a] (nM)
1	$CH_3CH_2CH_2$–	CH_3CH_2–	0.9 ± 0.2
2	$CH_3CH_2CH_2CH_2$–	CH_3CH_2–	0.98 ± 0.19
3	$CH_3CH_2CH_2CH_2CH_2$–	CH_3CH_2–	2.1 ± 0.3
4	$CH_3CH_2CH(CH_3)$–	CH_3CH_2–	18 ± 4.4
5	$(CH_3)_2CHCH_2$–	CH_3CH_2–	2.5 ± 1.7
6	$(CH_3)_3CCH_2$–	CH_3CH_2–	19 ± 3
7	Cyclopentyl–	CH_3CH_2–	7.9 ± 1.1
8	Cyclohexyl–	CH_3CH_2–	30 ± 11
9	Phenyl–	CH_3CH_2–	69 ± 21
10	$CH_3CH_2CH_2$–	H–	2.2 ± 0.4
11	$CH_3CH_2CH_2$–	NO_2–	0.87 ± 0.09
12	$CH_3CH_2CH_2$–	Br–	0.54 ± 0.23
13	$CH_3CH_2CH_2$–	Phenyl–	28 ± 9
14	$CH_3CH_2CH_2$–	Benzyl–	0.17 ± 0.01
15	CH_3CH_2–	Benzyl–	0.22 ± 0.05
16	Cyclopentyl–	Benzyl–	1.0 ± 0.3
17	$CH_3CH_2CH_2$–	Benzyl–CH_2–	0.42 ± 0.11
18	$CH_3CH_2CH_2$–	Benzyl–CH_2CH_2–	2.5 ± 0.3
19	$CH_3CH_2CH_2$–	2-Naphthyl–CH_2–	1.3 ± 0.2
20	$CH_3CH_2CH_2$–	4-Pyridyl–CH_2–	0.53 ± 0.11
21	$CH_3CH_2CH_2$–	3-Pyridyl–CH_2–	0.27 ± 0.09
22	$CH_3CH_2CH_2$–	2-Pyridyl–CH_2–	0.23 ± 0.07
23	Cyclopentyl–	3-Pyridyl–CH_2–	1.5 ± 0.2
24			17.1 ± 2.1
25		CH_3CH_2–	20 ± 5[b]
26		Benzyl–	1.4 ± 0.2[b]

[a] Each K_i value is the mean ± SD of three determinations.
[b] Previously published data.[11]

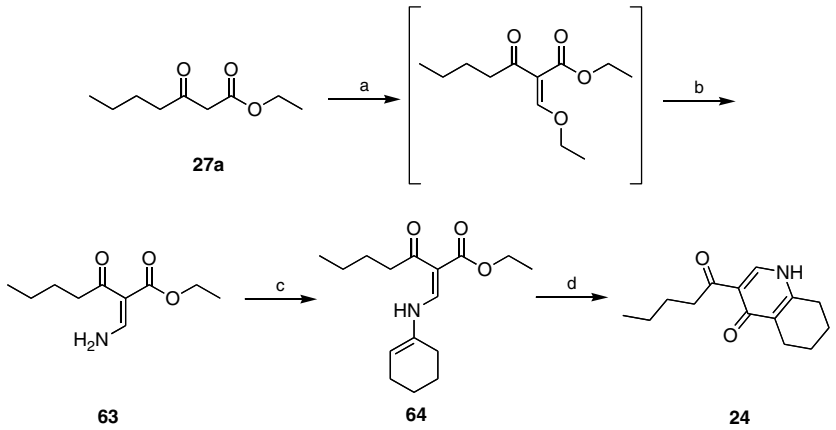
would be lost for the pyridyl derivatives when binding to the receptor. This is in accordance with the channel-like cavity that has been proposed to be situated in this area.[10]

A detailed comparison of the impact of the keto function on cortical tissue affinity can be made in those cases where affinity data of the corresponding 4-quinolones are available for the 3-acyl, 3-carboxylates and 3-carboxamides series. The butyl ketones **1** (0.9 nM) and **14** (0.17 nM) are comparable with the corresponding propyl esters, 1.8 nM and 0.17 nM, respectively.[12] However, it is obvious that the 3-acyl series is less sensitive to the length of the acyl carbon chain compared to the esters. A 3-butyryl instead of a 3-valeryl does practically not affect the affinity, **15** versus **14**, but the corresponding carbon chain decrease in the ester series gave rise to a compound with 8 times lower affinity (ethyl ester **26** vs its corresponding propyl ester).[12] Also, increasing the chain length from C_5 (**1**) to C_6 (**2**) and C_7 (**3**) has a minor effect in the acyl series while the affinity decreases 20 times for the esters.[12] The most potent 4-quinolone prepared so far is the amide (type B, Fig. 1) with R_1 = propyl and R_2 = benzyl, with the K_i value 0.048 nM. This is considerably more potent compared to the corresponding ester and ketone (both 0.17 nM), and a possible explanation is that the NH of the amide supports the binding by giving a (weak) hydrogen bond to H2/A3 (see Fig. 2).

Compound **24** was considered to be potentially interesting, as partly saturated derivatives in other types of compounds have displayed high-affinity at the benzodiazepine binding site.[5] However, the affinity was 8 times lower compared to its unsaturated parent compound (**10**), indicating that the electronic properties of **24**, and/or the small deviation from being planar, affect the affinity compared to the planar compound **10** in a negative way.

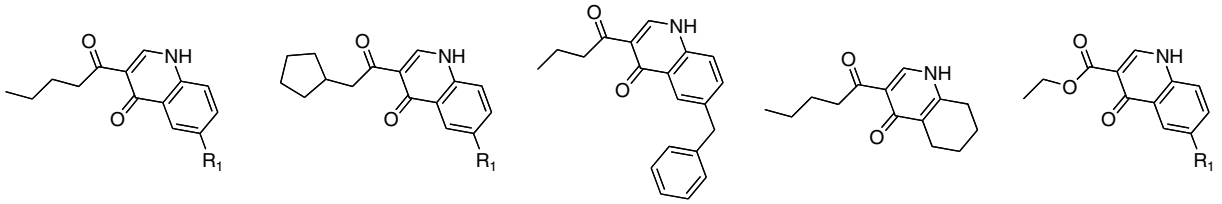
In order to assess the subtype specificity of the 3-acyl-4-quinolones, selected compounds were tested on recombinant $\alpha_1\beta_2\gamma_{2s}$, $\alpha_2\beta_2\gamma_{2s}$ and $\alpha_3\beta_2\gamma_{2s}$ receptor subtypes (results are shown in Table 2). We have previously shown that selectivity for α_1- over α_3-containing receptors can be obtained by including substituents interacting in the receptor 'interface' region as well as the L1 and L2 regions of the pharmacophore model (see Fig. 2).[12] One aim of the present study was therefore to reveal possible differences between α_1-, α_2-, and α_3-containing receptor subtypes focusing on the 'interface' region. All compounds investigated in this part of the study display a preference for $\alpha_1\beta_2\gamma_2$ over $\alpha_2\beta_2\gamma_2$ and $\alpha_3\beta_2\gamma_2$ receptor subtypes, respectively. The α_2/α_1 K_i ratio remained essentially the same for the compounds that were assayed, whereas the α_3/α_1 K_i ratio obviously depends on the substituent in position 6 of the 4-quinolone derivatives. The following discussion will therefore focus on differences between α_3- versus α_1-containing receptors.

In the ester series, the substitution of an ethyl to a benzyl group in the 6-position (**25** vs **26**) resulted in 3 times higher $\alpha_3/\alpha_1 K_i$ ratio as well as an affinity increase. The α_3/α_1 K_i ratio remained essentially unaffected for a similar ethyl to benzyl group substitution in the ketone series (**1** vs **14**). No significant subtype difference in the 'interface' region could be traced by bulkier 6-position substituents (**17**–**19**). This indicates that the three different subtypes all accept bulky substituents to the same extent in the 'interface' region, at least when it comes to 3-valeryl-4-quinolones. The pyridyl derivative **20** displays the same α_3/α_1 K_i ratio as its parent benzyl compound **14**. However, and interestingly, the other two pyridyl isomers **21** and **22** show a 3-fold enhanced α_3/α_1 K_i ratio compared to **20**. This indicates that there are differences between the two receptor subtypes in the 'interface' region, which could be included in a future subtype specific pharmacophore model. The fused benzene ring of the 4-quinolones interact, according to pharmacophore model, with the same receptor region as the saturated ring structures in imidazopyridopyrimidinone- and pyrazolo-3-pyridinone derivatives that display functional selectivity at receptor subtypes.[5,26] Therefore, we were interested in investigating the affinity of a partly saturated 4-quinolone derivative (**10** vs **24**). The affinity decreased for **24** at both α_1- and α_3-containing receptors, and the α_3/α_1 K_i ratio decreased indicating additional differences between the receptor subtypes. Previous research has shown that bulky substituents in the L2 region result in an increase of the α_3/α_1 K_i ratio.[6,12] The less bulky propyl ketone **15** displayed,



Scheme 4. Reagents and conditions: (a) triethyl orthoformate, acetic anhydride, 100 °C, over night; (b) NH_3, EtOH, rt, 5 h; (c) cyclohexanon, *p*-TsOH, toluene, Dean-Stark conditions, 24 h; (d) diphenyl ether, reflux, 30 min.

Table 2
The affinity of selected 4-quinolones tested on ^{3}H-flumazenil binding to $\alpha_1\beta_2\gamma_{2s}$, $\alpha_2\beta_2\gamma_{2s}$ and $\alpha_3\beta_2\gamma_{2s}$ $GABA_A$ receptor subtypes



1, **10**, **14**, **17**-**22** **7**, **16**, **23** **15** **24** **25**, **26**

Substance	R_1	$K_i\alpha_1$ (nM)	$K_i\alpha_2$ (nM)	$K_i\alpha_3$ (nM)	K_i ratio α_2/α_1	K_i ratio α_3/α_1
1	CH_3CH_2–	0.38 ± 0.05[a]	1.6 ± 0.4[a]	2.8 ± 0.1[a]	4	7
7	CH_3CH_2–	5.1 ± 0.8[a]	31 ± 10[a]	74 ± 24[a]	6	15
10	H–	0.97 ± 0.15[a]	Not tested	9.2 ± 2.3[a]	–	9
14	Benzyl–	0.12 ± 0.04[a]	0.40 ± 0.15[a]	0.61 ± 0.11[a]	3	5
15		0.12 ± 0.05[a]	0.81 ± 0.13[a]	1.56 ± 0.96[a]	7	13
16	Benzyl–	0.51 ± 0.05[a]	Not tested	13 ± 2[a]	–	25
17	Benzyl-CH_2–	0.53 ± 0.31[a]	1.7 ± 0.7[a]	1.1 ± 0.1[a]	3	2
18	Benzyl-CH_2CH_2–	3.2 ± 1.5[a]	16 ± 8[a]	14.3 ± 9.6[a]	5	4
19	(2-Naphthyl)CH_2–	2.7 ± 1.0[a]	22 ± 13[a]	8.8 ± 5.5[a]	8	3
20	(4-Pyridyl)CH_2–	0.39 ± 0.20[a]	2.7 ± 1.3[a]	2.1 ± 0.8[a]	7	5
21	(3-Pyridyl)CH_2–	0.12 ± 0.02[a]	Not tested	2.2 ± 0.2[a]	–	18
22	(2-Pyridyl)CH_2–	0.14 ± 0.02[a]	Not tested	2.2 ± 0.6[a]	–	16
23	(3-Pyridyl)CH_2–	0.88 ± 0.27[a]	Not tested	23 ± 2[a]	–	26
24		11 ± 1[a]	Not tested	51 ± 9[a]	–	5
25	CH_3CH_2–	5.9 ± 0.4[a]	Not tested	52 ± 8[a]	–	9
26	Benzyl–	0.27 ± 0.06[a,b]	Not tested	7.2 ± 0.03[a,b]	–	27

[a] Each K_i value is the mean ± SD of three determinations.
[b] Previously published data.[11]

however, a higher α_3/α_1 K_i ratio than the corresponding butyl ketone **14**. Also the cyclopentylketone **7** showed a higher α_1 selectivity than corresponding butylketone **1**.

In order to investigate if substances with a 3-cyclopentyl methylene ketone and a bulky substituent in position 6 could display synergetic effects, for example, higher subtype selectivity than **7**, compounds **16** and **23** were synthesized and tested. This was apparently a successful approach, resulting in with two high-affinity ligands that display a α_3/α_1 K_i ratio of 25 and 26, respectively, and this path will be further investigated.

4. Conclusion

The affinity of the 3-acyl-4-quinolones for the benzodiazepine binding site of the $GABA_A$ receptor is to a large extent similar to that of the corresponding esters and amides studied previously, in spite of the differences in the electronic properties of the carbonyl function in the three compound types. Nevertheless, set of compounds prepared and assayed in this investigation have permitted additional characterization of the 'interface', as defined in the pharmacophore model. The decreased affinity that is displayed by **18** and **19**, with bulky 6-substituents, indicate the presence of receptor essential volumes (steric repulsive interactions) further out in the 'interface'. Several substances have been tested on the $\alpha_1\beta_2\gamma_{2s}$, $\alpha_2\beta_2\gamma_{2s}$ and $\alpha_3\beta_2\gamma_{2s}$ $GABA_A$ receptor subtypes. Based on these results, **16** and **23** were designed to display selectivity for α_1- over α_3-containing receptors, and were shown to have strong affinity at the $\alpha_1\beta_2\gamma_{2s}$ subtype ($K_i < 1$ nM) and α_3/α_1 K_i ratios of 25 and 26, respectively. As the ketones can be expected to be metabolically more stable than the corresponding esters or amides, selected 4-quinolones from present study are currently undergoing in vivo studies.

5. Experimental

Reagents and solvents (except THF) were used from commercial sources without purification. THF was distilled from sodium/benzophenone prior to use. ^{1}H and ^{13}C NMR were recorded at room temperature with a Bruker ARX300 or a Bruker DRX400 spectrometer. The spectra were recorded in $CDCl_3$, DMSO-d_6, and CD_3OD, and the solvent signals (7.27 and 77.0, 2.50 and 39.5 or 3.31 and 49.0 ppm, respectively) were used as reference. The raw data were transformed and the spectra were evaluated with the standard Bruker UXNMR software (rev. 941001). Analytical thin-layer chromatography (TLC) was performed on Kiselgel 60 F_{254} plates (Merck). Column chromatography was performed on SiO_2 (Matrex LC-gel: 60A, 35–70 MY, Grace). Melting points (uncorrected) were determined with a Reichert microscope. EI mass spectra were recorded at 70 eV with a Jeol SX102 spectrometer and ESI spectra were recorded with Micromass Q-TOF Micro. The purity of the assayed compounds was verified with ^{1}H NMR and HPLC, and only used if more than 98% pure.

5.1. 6-Ethyl-3-pentanoyl-4-quinolone (1)

41 (vide infra) (0.134 g, 0.442 mmol) was dissolved in diphenyl ether (2 ml). The mixture was refluxed for 1 h and then allowed to cool to rt. Petroleum ether was added and the resulting crystals were collected and washed with a large amount of petroleum ether. The crystals were purified by trituration with diethyl ether yielding **1** (0.068 g, 0.264 mmol, 60%), a white solid (mp: 206–208 °C). ^{1}H NMR (MeOD) δ 0.96 (3H, t, J = 7.3), 1.30 (3H, t, J = 7.6), 1.42 (2H, m), 1.65 (2H, m), 2.80 (2H, q, J = 7.6), 3.19 (2H, t, J = 7.3), 7.51 (1H, d, J = 8.4), 7.63 (1H, dd, J = 8.5, 2.0), 8.16 (1H, m), 8.54 (1H, s); ^{13}C NMR (DMSO-d_6) δ 13.9, 15.5, 22.0, 26.1, 27.9, 42.2, 117.3, 118.8, 123.8, 127.8, 132.7, 137.2, 140.6, 143.7, 175.0, 199.2. HRMS (ESI): for $C_{16}H_{19}NO_2Na$ calcd: 280.1313 $[MNa]^+$; found: 280.1320.

5.2. 6-Ethyl-3-hexanoyl-4-quinolone (2)

The title compound was prepared and purified according to the procedure described for **1**, starting from **42** (vide infra). The reaction yielded **2** (57%), a white solid (mp: 209–211 °C). ^{1}H NMR (MeOD) δ 0.92 (3H, t, 7.1), 1.30 (3H, t, J = 7.6), 1.37 (4H, m), 1.67 (2H, m), 2.80 (2H, q, J = 7.6), 3.17 (2H, t, J = 7.4), 7.50 (1H, d, J = 8.4), 7.62 (1H, dd, J = 8.4, 1.9), 8.15 (1H, d, J = 1.9), 8.54 (1H, s); ^{13}C NMR (MeOD) δ 14.4, 16.0, 23.7, 25.3, 29.6, 32.9, 44.2, 119.1, 119.8, 125.2, 129.2, 134.6, 138.8, 143.4, 145.3, 178.2, 202.6. HRMS (ESI): for $C_{17}H_{22}NO_2$ calcd: 272.1651 $[MH]^+$; found: 272.1662.

5.3. 6-Ethyl-3-heptanoyl-4-quinolone (3)

The title compound was prepared and purified according to the procedure described for **1**, starting from **43** (vide infra). The reaction yielded **3** (46%), a white solid (mp: 208–210 °C). ^{1}H NMR (MeOD) δ 0.90 (3H, t, J = 6.8), 1.33 (9H, m), 1.66 (2H, m), 2.80 (2H, q, J = 7.6), 3.18 (2H, t, J = 7.4), 7.50 (1H, d, J = 8.4), 7.62 (1H, dd, J = 8.4, 1.9), 8.15 (1H, d, J = 1.9), 8.54 (1H, s); ^{13}C NMR (MeOD) δ 14.4, 16.0, 23.7, 25.5, 29.6, 30.3, 33.0, 44.2, 119.1, 119.8, 125.2, 129.2, 134.6, 138.8, 143.4, 145.3, 178.2, 202.5. HRMS (ESI): for $C_{18}H_{23}NO_2Na$ calcd: 308.1626 $[MNa]^+$; found: 308.1631.

5.4. 6-Ethyl-3-(3-methylpentanoyl)-4-quinolone (4)

The title compound was prepared and purified according to the procedure described for **1**, starting from **44** (vide infra). The reaction yielded **4** (47%), a white solid (mp: 183–184 °C). ^{1}H NMR (MeOD) δ 0.93 (6H, m), 1.28 (4H, m), 1.44 (1H, m), 2.02 (1H, m), 2.80 (2H, q, J = 7.5), 2.96 (1H, m), 3.23 (1H, m), 7.51 (1H, d, J = 8.4), 7.62 (1H, d, J = 8.4), 8.16 (1H, s), 8.53 (1H, s); ^{13}C NMR (MeOD) δ 11.8, 16.0, 19.9, 29.6, 30.9, 32.4, 51.2, 119.5, 119.8, 125.3, 129.2, 134.6, 138.8, 143.4, 145.4, 178.1, 202.5. HRMS (ESI): for $C_{17}H_{22}NO_2$ calcd: 272.1651 $[MH]^+$; found: 272.1646.

5.5. 6-Ethyl-3-(4-methylpentanoyl)-4-quinolone (5)

The title compound was prepared and purified according to the procedure described for **1**, starting from **45** (vide infra). The reaction yielded **5** (35%), a white solid (mp: 196–198 °C). ^{1}H NMR (MeOD) δ 0.95 (6H, d, J = 6.5), 1.30 (3H, t, J = 7.6), 1.55 (2H, m), 1.64 (1H, m), 2.80 (2H, q, J = 7.6), 3.20 (2H, t, J = 7.6), 7.50 (1H, d, J = 8.4), 7.61 (1H, d, J = 8.4), 8.16 (1H, s), 8.53 (1H, s); ^{13}C NMR (MeOD) δ 16.0, 22.9, 22.9, 29.2, 29.6, 34.5, 42.3, 119.1, 119.8, 125.2, 129.2, 134.6, 138.8, 143.4, 145.3, 178.2, 202.8. HRMS (ESI): for $C_{17}H_{22}NO_2$ calcd: 272.1651 $[MH]^+$; found: 272.1657.

5.6. 3-(4,4-Dimethylpentanoyl)-6-ethyl-4-quinolone (6)

The title compound was prepared and purified according to the procedure described for **1**, starting from **46** (vide infra). The reaction yielded **6** (34%), a white solid (mp: 217–219 °C). ^{1}H NMR (DMSO-d_6) δ 0.91 (9H, s), 1.23 (3H, t, J = 7.5), 1.48 (2H, m), 2.74 (2H, q, J = 7.5), 3.09 (2H, m), 7.55 (1H, d, J = 8.4), 7.59 (1H, d, J = 8.4), 8.06 (1H, s), 8.46 (1H, s), 12.44 (1H, s); ^{13}C NMR (DMSO-d_6) δ 15.5, 27.9, 29.2, 29.2, 29.2, 29.9, 37.7, 38.2, 117.3, 118.8, 123.9, 127.8, 132.7, 137.2, 140.6, 143.7, 175.0, 199.8. HRMS (ESI): for $C_{18}H_{24}NO_2$ calcd: 286.1807 $[MH]^+$; found: 286.1808.

5.7. 3-(Cyclopentylacetyl)-6-ethyl-4-quinolone (7)

The title compound was prepared and purified according to the procedure described for **1**, starting from **47** (vide infra). The reaction yielded **7** (50%), a white solid (mp: 209–211 °C). ^{1}H NMR (MeOD) δ 1.21 (2H, m), 1.30 (3H, t, J = 7.6), 1.60 (4H, m), 1.84 (2H, m), 2.35 (1H, m), 2.80 (2H, q, J = 7.6), 3.22 (2H, d, J = 7.1), 7.51 (1H, d, J = 8.4), 7.62 (1H, dd, J = 8.4, 1.9), 8.16 (1H, d, J = 1.9), 8.53 (1H, s); ^{13}C NMR (MeOD) δ 16.0, 26.0, 26.0, 29.6, 33.7, 33.7, 37.2, 50.4, 119.3, 119.8, 125.2, 129.3, 134.6, 138.8, 143.4, 145.3, 178.2, 202.3. HRMS (ESI): for $C_{18}H_{22}NO_2$ calcd: 284.1651 $[MH]^+$; found: 284.1649.

5.8. 3-(Cyclohexylacetyl)-6-ethyl-4-quinolone (8)

The title compound was prepared and purified according to the procedure described for **1**, starting from **48** (vide infra). The reaction yielded **8** (68%), a white solid (mp: 229-231 °C). ^{1}H NMR (DMSO-d_6) δ 0.95 (2H, m), 1.17 (6H, m), 1.65 (5H, m), 1.82, (1H, s), 2.73 (2H, q, J = 7.6), 2.99 (2H, d, J = 6.8), 7.56 (2H, m), 8.04 (1H, s), 8.46 (1H, s); ^{13}C NMR (DMSO-d_6) δ 15.5, 25.8, 25.8, 25.9, 27.9, 32.9, 32.9, 33.7, 49.9, 117.6, 118.9, 123.9, 127.8, 132.7, 137.3, 140.6, 143.8, 174.9, 198.7. HRMS (ESI): for $C_{19}H_{24}NO_2$ calcd: 298.1807 $[MH]^+$; found: 298.1796.

5.9. 6-Ethyl-3-(phenylacetyl)-4-quinolone (9)

The title compound was prepared and purified according to the procedure described for **1**, starting from **49** (vide infra). The reaction yielded **9** (59%), a white solid (mp: 227–229 °C). ^{1}H NMR (MeOD) δ 1.31 (3H, t, J = 7.6), 2.80 (2H, q, J = 7.6), 4.54 (2H, s), 7.22 (5H, m), 7.49 (1H, d, J = 8.5), 7.61 (1H, dd, J = 8.4, 1.6) 8.18 (1H, d, J = 1.6), 8.51 (1H, s); ^{13}C NMR (MeOD) δ 16.0, 29.6, 49.9, 118.8, 119.9, 125.3, 127.5, 129.2, 129.3, 129.3, 130.9, 130.9,

134.6, 137.0, 138.8, 143.5, 145.6, 178.2, 199.9. HRMS (ESI): for $C_{19}H_{18}NO_2$ calcd: 292.1338 $[MH]^+$; found: 292.1338.

5.10. 3-Pentanoyl-4-quinolone (10)

The title compound was prepared and purified according to the procedure described for **1**, starting from **50** (vide infra). The reaction yielded **10** (63%), a white solid (mp: 225–227 °C). ^{1}H NMR (MeOD) δ 0.95 (3H, t, J = 7.4), 1.42 (2H, m), 1.65 (2H, m), 3.18 (2H, t, J = 7.4), 7,48 (1H, ddd, J = 8.2, 7.1, 1.1), 7.57 (1H, d, J = 8.2), 7.74 (1H, ddd, J = 8.2, 7.1, 1.4), 8.33 (1H, dd, J = 8.2, 1.4), 8.57 (1H, s); ^{13}C NMR (MeOD) δ 14.4, 23.7, 27.7, 43.9, 119.4, 119.8, 126.6, 127.2, 129.3, 134.1, 140.6, 145.8, 178.2, 202.4.HRMS (ESI): for $C_{14}H_{16}NO_2$ calcd: 230.1181 $[MH]^+$; found: 230.1189.

5.11. 6-Nitro-3-pentanoyl-4-quinolone (11)

A solution of 68% HNO_3 (52 μl, 0.785 mmol) in 1.5 ml concd H_2SO_4 was added to a solution of **10** (150 mg, 0.654 mmol) in 1.5 ml H_2SO_4 at −10 °C. The mixture was stirred at −10 °C for 4 h whereupon it was poured on ice. The mixture was extracted with diethyl ether, and the extract was dried with $MgSO_4$, and then concentrated. The crude product was purified by chromatography ($CHCl_3$/EtOH, 50:1) followed by recrystallization from ethanol to give **11** (76 mg, 0.277 mmol, 42%), a white solid (mp: 278–280 °C). ^{1}H NMR (DMSO-d_6) δ 0.89 (3H, t, J = 7.4), 1.33 (2H, sex, J = 7.4), 1.55 (2H, quint, J = 7.4), 3.08 (2H, t, J = 7.4), 7.80 (1H, d, J = 9.1), 8.46 (1H, dd, J = 9.1, 2.7), 8.6 (1H, s), 8.90 (1H, d, J = 2.7); ^{13}C NMR (DMSO-d_6) δ 13.9, 22.0, 25.9, 42.2, 118.7, 120.8, 122.0, 126.7, 127.3, 143.0, 143.8, 145.6, 174.4, 198.7. HRMS (ESI): for $C_{14}H_{15}N_2O_4$ calcd: 275.1032 $[MH]^+$; found: 275.1020.

5.12. 6-Bromo-3-pentanoyl-4-quinolone (12)

The title compound was prepared and purified according to the procedure described for **1**, starting from **51** (vide infra). The reaction yielded **12** (80%), a white solid (mp: 295–297 °C). ^{1}H NMR (DMSO-d_6) δ 0.89 (3H, t, J = 7.3), 1.32 (2H, m), 1.55 (2H, m), 3.08 (2H, t, J = 7.3), 7.60 (1H, d, J = 8.8), 7.87 (1H, dd, J = 8.8, 2.3), 8.29 (1H, d, J = 2.3), 8.54 (1H, s); ^{13}C NMR (DMSO-d_6) δ 13.9, 22.0, 25.9, 42.2, 117.6, 117.8, 121.5, 127.9, 129.3, 135.2, 138.1, 144.6, 173.7, 198.9. HRMS (ESI): for $C_{14}H_{151}NO_2Br$ calcd: 308.0286 $[MH]^+$; found: 308.0296.

5.13. 3-Pentanoyl-6-phenyl-4-quinolone (13)

The title compound was prepared and purified according to the procedure described for **1**, starting from **52** (vide infra). The reaction yielded **13** (82%), a white solid (mp: 267–269 °C). ^{1}H NMR (DMSO-d_6) δ 0.90 (3H, t, J = 7.3), 1.34 (2H, m), 1.57 (2H, m), 3.13 (2H, t, J = 7.4), 7.41 (1H, m), 7.52 (2H, m), 7.74 (3H, m), 8.06 (1H, dd, J = 8.5, 2.1), 8.46 (1H, d, J = 2.1), 8.54 (1H, s); ^{13}C NMR (DMSO-d_6) δ 14.0, 22.1, 26.1, 42.3, 117.7, 119.8, 123.2, 123.2, 126.8, 127.8, 128.1, 129.2, 129.2, 131.2, 136.7, 138.5, 139.2, 144.2, 175.1, 199.2. HRMS (ESI): for $C_{20}H_{20}NO_2$ calcd: 306.1494 $[MH]^+$; found: 306.1508.

5.14. 6-Benzyl-3-pentanoyl-4-quinolone (14)

The title compound was prepared and purified according to the procedure described for **1**, starting from **53** (vide infra). The reaction yielded **14** (72%), a white solid (mp: 209–211 °C). ^{1}H NMR (DMSO-d_6) δ 0.88 (3H, t, J = 7.4), 1.32 (2H, sex, J = 7.4), 1.54 (2H, quint, J = 7.4), 3.08 (2H, t, J = 7.4), 4.08 (2H, s), 7.26 (5H, m), 7.55 (1H, d, J = 8.4), 7.61 (1H, dd, J = 8.4, 2.0), 8.04 (1H, d, J = 2.0), 8.46 (1H, d, J = 6.7), 12.46 (1H, d, J = 6.7); ^{13}C NMR (DMSO-d_6) δ 13.9, 22.0, 26.1, 40.6, 42.2, 117.4, 119.0, 125.1, 126.1, 127.8, 128.5, 128.5, 128.8, 128.8, 133.4, 137.4, 138.3, 140.9, 143.8, 174.9, 199.1. HRMS (ESI): for $C_{21}H_{22}NO_2$ calcd: 320.1651 $[MH]^+$; found: 320.1655.

5.15. 6-Benzyl-3-butyryl-4-quinolone (15)

The title compound was prepared and purified according to the procedure described for **1**, starting from **54** (vide infra). The reaction yielded **15** (71%), a white solid (mp: 230–232 °C). ^{1}H NMR (DMSO-d_6) δ 0.87 (3H, t, J = 7.3), 1.54 (2H, sex, J = 7.3), 3.02 (2H, t, J = 7.3), 4.05 (2H, s), 7.17 (1H, m), 7.25 (4H, m), 7.52 (1H, d, J = 8.4), 7.57 (1H, dd, J = 8.4, 1.8), 8.01 (1H, d, J = 1.8), 8.44 (1H, s), 12.44 (1H, br s); ^{13}C NMR (DMSO-d_6) δ 13.9, 17.3, 40.6, 44.5, 117.4, 119.0, 125.1, 126.1, 127.8, 128.5, 128.5, 128.8, 128.8, 133.4, 137.4, 138.3, 140.9, 143.8, 174.9, 199.0. HRMS (ESI): for $C_{20}H_{20}NO_2$ calcd: 306.1494 $[MH]^+$; found: 306.1493.

5.16. 6-Benzyl-3-(cyclopentylacetyl)-4-quinolone (16)

The title compound was prepared and purified according to the procedure described for **1**, starting from **55** (vide infra). The reaction yielded **16** (88%), a white solid (mp: 215–217 °C). ^{1}H NMR (DMSO-d_6) δ 1.12 (2H, m), 1.46 (2H, m), 1.57 (2H, m), 1.74 (2H, m), 2.24 (1H, m), 3.11 (2H, d, J = 7.1), 4.08 (2H, s), 7.21 (1H, m), 7.28 (4H, m), 7.55 (1H, d, J = 8.4), 7.61 (1H, dd, J = 8.4, 2.0), 8.03 (1H, d, J = 2.0), 8.46 (1H, s), 12.5 (1H, br s); ^{13}C NMR (DMSO-d6) δ 24.5, 24.5, 32.2, 32.2, 35.4, 40.6, 48.6, 117.5, 119.0, 125.0, 126.1, 127.8, 128.5, 128.5, 128.8, 128.8, 133.4, 137.4, 138.2, 140.9, 143.8, 174.9, 198.9. HRMS (ESI): for $C_{23}H_{24}NO_2$ calcd: 346.1807 $[MH]^+$; found: 346.1795.

5.17. 3-Pentanoyl-6-(2-phenylethyl)-4-quinolone (17)

The title compound was prepared and purified according to the procedure described for **1**, starting from **56** (vide infra). The reaction yielded **17** (67%), a white solid (mp: 223–225 °C). ^{1}H NMR (DMSO-d_6) δ 0.89 (3H, t, J = 7.4), 1.33 (2H, sex, J = 7.4), 1.55 (2H, quint, J = 7.4), 2.93 (2H, m), 3.01 (2H, m), 3.09 (2H, t, J = 7.4), 7.17 (1H, m), 7.25 (4H, m), 7.53 (1H, d, J = 8.4), 7.59 (1H, dd, J = 8.4, 1.8), 8.06 (1H, d, J = 1.8), 8.46 (1H, d, J = 5.9), 12.44 (1H, d, J = 5.9); ^{13}C NMR (DMSO-d_6) δ 13.9, 22.1, 26.1, 36.7, 36.9, 42.2, 117.3, 118.7, 124.8, 125.9, 127.7, 128.2, 128.2, 128.4, 128.4, 133.2, 137.3, 138.4, 141.2, 143.7, 175.0, 199.2. HRMS (ESI): for $C_{22}H_{24}NO_2$ calcd: 334.1807 $[MH]^+$; found: 334.1819.

5.18. 3-Pentanoyl-6-(3-phenylpropyl)-4-quinolone (18)

The title compound was prepared and purified according to the procedure described for **1**, starting from **57** (vide infra). The reaction yielded **18** (41%), a white solid (mp: 171-173 °C). ^{1}H NMR (DMSO-d_6) δ 0.88 (3H, t, J = 7.3), 1.32 (2H, sex, J = 7.3), 1.54 (2H, quint, J = 7.3), 1.92 (2H, quint, J = 7.5), 2.60 (2H, t, J = 7.5), 2.73 (2H, t, J = 7.5), 3.09 (2H, t, J = 7.3), 7.23 (5H, m), 7.54 (1H, d, J = 8.4), 7.58 (1H, dd, J = 8.4, 1.7), 8.03 (1H, br s), 8.46 (1H, d, J = 6.5), 12.48 (1H, d, J = 6.5); ^{13}C NMR (DMSO-d_6) δ 14.1, 22.2, 26.2, 32.7, 34.5, 34.7, 42.3, 117.5, 119.0, 124.7, 125.9, 127.9, 128.4, 128.4, 128.4, 128.4, 133.3, 137.4, 139.0, 142.0, 143.9, 175.2, 199.5. HRMS (ESI): for $C_{23}H_{25}NO_2Na$ calcd: 370.1783 $[MNa]^+$; found: 370.1792.

5.19. 6-(2-Naphthylmethyl)-3-pentanoyl-4-quinolone (19)

The title compound was prepared and purified according to the procedure described for **1**, starting from **58** (vide infra). The reaction yielded **19** (65%), a white solid (mp: 222–224 °C). ^{1}H NMR (DMSO-d_6) δ 0.86 (3H, t, J = 7.4), 1.30 (2H, sex, J = 7.4), 1.52 (2H,

quint, J = 7.4), 3.05 (2H, t, J = 7.4), 4.25 (2H, s), 7.39 (1H, dd, J = 8.4, 1.5), 7.47 (2H, m), 7.56 (1H, d, J = 8.4), 7.66 (1H, dd, J = 8.4, 1.9), 7.78 (1H, br s), 7.85 (3H, m), 8.07 (1H, d, J = 1.5), 8.45 (1H, s); ^{13}C NMR (DMSO-d_6) δ 14.0, 22.2, 26.2, 40.9, 42.3, 117.5, 119.3, 125.3, 125.7, 126.3, 126.9, 127.5, 127.6, 127.7, 127.9, 128.2, 131.8, 133.3, 133.7, 137.6, 138.3, 138.6, 144.0, 175.1, 199.4. HRMS (ESI): for $C_{25}H_{23}NO_2Na$ calcd: 392.1626 $[MNa]^+$; found: 392.1638.

5.20. 3-Pentanoyl-6-(pyridin-4-yl-methyl)-4-quinolone (20)

The title compound was prepared and purified according to the procedure described for **1**, starting from **59** (vide infra). The reaction yielded **20** (47%), a white solid (mp: 152–154 °C). ^{1}H NMR (MeOD) δ 0.95 (3H, t, J = 7.4), 1.40 (2H, sex, J = 7.4), 1.64 (2H, quint, J = 7.4), 3.16 (2H, t, J = 7.4), 4.19 (2H, s), 7.32 (2H, d, J = 5.8), 7.55 (1H, d, J = 8.5), 7.62 (1H, dd, J = 8.5, 1.6), 8.20 (1H, d, J = 1.6), 8.43 (2H, d, J = 5.8), 8.55 (1H, s); ^{13}C NMR (MeOD) δ 14.5, 23.8, 27.8, 41.7, 44.1, 119.5, 120.6, 126.1, 126.1, 127.1, 129.5, 135.3, 138.4, 139.6, 145.9, 150.3, 150.3, 152.8, 178.0, 202.5. HRMS (ESI): for $C_{20}H_{21}N_2O_2$ calcd: 321.1603 $[MH]^+$; found: 321.1589.

5.21. 3-Pentanoyl-6-(pyridin-3-yl-methyl)-4-quinolone (21)

The title compound was prepared and purified according to the procedure described for **1**, starting from **60** (vide infra). The reaction yielded **21** (72%), a white solid (mp: 197–199 °C). ^{1}H NMR (DMSO-d_6) δ 0.88 (3H, t, J = 7.4), 1.32 (2H, sex, J = 7.4), 1.54 (2H, quint, J = 7.4), 3.08 (2H, t, J = 7.4), 4.12 (2H, s), 7.33 (1H, dd, J = 7.8, 4.8), 7.57 (1H, d, J = 8.4), 7.64 (2H, m), 8.05 (1H, d, J = 1.7), 8.43 (1H, d, J = 4.8), 8.47 (1H, s), 8.55 (1H, s), 12.48 (1H, br s); ^{13}C NMR (DMSO-d_6) δ 13.9, 22.1, 26.1, 37.6, 42.2, 117.5, 119.2, 123.7, 125.1, 127.9, 133.3, 136.3, 136.5, 137.5, 137.5, 143.9, 147.5, 149.9, 174.9, 199.2. HRMS (ESI): for $C_{20}H_{21}N_2O_2$ calcd: 321.1603 $[MH]^+$; found: 321.1592.

5.22. 3-Pentanoyl-6-(pyridin-2-yl-methyl)-4-quinolone (22)

The title compound was prepared and purified according to the procedure described for **1**, starting from **61** (vide infra). The reaction yielded **22** (55%), a white solid (mp: 191-193 °C). ^{1}H NMR (DMSO-d_6) δ 0.88 (3H, t, J = 7.4), 1.32 (2H, sex, J = 7.4), 1.54 (2H, quint, J = 7.4), 3.08 (2H, t, J = 7.4), 4.22 (2H, s), 7.22 (1H, dd, J = 7.4, 4.9), 7.33 (1H, d, J = 7.8), 7.56 (1H, d, J = 8.4), 7.65 (1H, dd, J = 8.4, 1.8), 7.72 (1H, dt, J = 7.7, 1.7), 8.08 (1H, d, J = 1.8), 8.46 (1H, d, J = 6.6), 8.49 (1H, m), 12.47 (1H, d, J = 6.6); ^{13}C NMR (DMSO-d_6) δ 13.9, 22.0, 26.1, 42.2, 43.3, 117.4, 119.0, 121.6, 123.2, 125.4, 127.8, 133.6, 136.8, 136.8, 137.5, 143.8, 149.2, 160.2, 174.9, 199.2. HRMS (ESI): for $C_{20}H_{21}N_2O_2$ calcd: 321.1603 $[MH]^+$; found: 321.1587.

5.23. 3-(Cyclopentylacetyl)-6-(pyridin-3-yl-methyl)-4-quinolone (23)

The title compound was prepared and purified according to the procedure described for **1**, starting from **62** (vide infra). The reaction yielded **23** (49%), a white solid (mp: 184–186 °C). ^{1}H NMR (DMSO-d_6) δ 1.11 (2H, m), 1.46 (2H, m), 1.57 (2H, m), 1.74 (2H, m), 2.24 (1H, m), 3.11 (2H, d, J = 7.1), 4.12 (2H, s), 7.34 (1H, dd, J = 7.8, 4.8), 7.57 (1H, d, J = 8.4), 7.63 (1H, dd, J = 8.4, 1.9), 7.67 (1H, dt, J = 7.8, 1.3), 8.04 (1H, d, J = 1.9), 8.44 (1H, dd, J = 4.8, 1.3), 8.46 (1H, d, J = 6.6), 8.55 (1H, d, J = 1.3), 12.49 (1H, d, J = 6.6); ^{13}C NMR (DMSO-d_6) δ 24.5, 24.5, 32.2, 32.2, 35.4, 37.6, 48.6, 117.6, 119.2, 123.7, 125.1, 127.9, 133.3, 136.5, 136.5, 137.4, 137.5, 143.9, 147.4, 149.8, 174.9, 198.9. HRMS (ESI): for $C_{22}H_{23}N_2O_2$ calcd: 347.1760 $[MH]^+$; found: 347.1751.

5.24. 3-Pentanoyl-5,6,7,8-tetrahydro-4-quinolone (24)

64 (vide infra) (94 mg, 0.337 mmol) was refluxed in 1 ml of diphenyl ether for 30 min. The mixture was concentrated under reduced pressure, whereupon the crude product was purified by chromatography (petroleum ether/EtOAc, 30:1–1:1) yielding **24** (25 mg, 0.107 mmol, 32%), a white solid (mp: 174–176 °C). ^{1}H NMR ($CDCl_3$) δ 0.96 (3H, t, J = 7.4), 1.42 (2H, m), 1.73 (2H, m), 1.83 (4H, m), 2.67 (2H, t, J = 6.1), 2.87 (2H, t, J = 6.1), 3.01 (2H, t, J = 7.4), 8.70 (1H, s); ^{13}C NMR ($CDCl_3$) δ 13.8, 21.5, 21.7, 22.2, 22.4, 26.6, 32.2, 38.4, 115.1, 122.4, 147.6, 161.3, 167.5, 206.1. HRMS (ESI): for $C_{14}H_{20}NO_2$ calcd: 234.1494 $[MH]^+$; found: 234.1487.

5.25. Ethyl 3-oxoheptanoate (27a)

n-Butyllithium (12.29 ml 2.5 M in hexane, 30.73 mmol) was added slowly to a −78 °C solution of monoethyl malonate (2.030 g, 15.37 mmol) in 40 ml anhydrous THF, while allowing the temperature to rise to −10 °C near the end of the addition. After 10 min at −10 °C, the mixture was cooled to −78 °C and valeryl chloride was added dropwise. Cooling was removed and after 10 min the reaction was quenched with 1 M HCl (aq) (30 ml). The mixture was extracted with diethyl ether (60 ml). The organic extract was washed with satd $NaHCO_3$ (aq) (2× 20 ml), H_2O (20 ml) and brine (20 ml), then dried with $MgSO_4$. Evaporation of the solvent yielded **27a** (1.479 g, 8.59 mmol, 95%), as a colourless oil. Characterization data agreed with previous publication.[15]

5.26. Ethyl 3-oxooctanoate (27b)

Ethyl acetoacetate (0.204 g, 1.57 mmol) was added dropwise to a 0 °C solution of NaH (69 mg with 60% mineral oil, 1.72 mmol) in 5 ml anhydrous THF. After 20 min, *tert*-butyllithium (1.05 ml 1.5 M soln in pentane, 1.57 mmol) was added dropwise and the resulting mixture was stirred at 0 °C for 30 min. 1-Iodobutane in 5 ml of anhydrous THF was added dropwise to the dianion solution whereupon cooling was removed. After approximately 15 min, the reaction was quenched by 10 ml of satd NH_4Cl (aq), whereupon the mixture was extracted with diethyl ether (3× 15 ml). The combined organic extract was washed with brine (15 ml) and dried with $MgSO_4$. The crude product was purified by chromatography (petroleum ether/EtOAc, 30:1–20–1), yielding **27b** (0123 g, 0.660 mmol, 42%) as a colourless oil. Characterization data agreed with previous publication.[27]

5.27. Ethyl 3-oxononanoate (27c)

The title compound was prepared and purified according to the procedure described for **27b**, using 1-bromopentane instead of 1-iodobutane. The reaction yielded **27c** (30%), a colourless oil. Characterization data agreed with previous publication.[28]

5.28. Ethyl 5-methyl-3-oxoheptanoate (27d)

The title compound was prepared and purified according to the procedure described for **27b**, using 2-iodobutane instead of 1-iodobutane. The reaction yielded **27d** (24%), a colourless oil. ^{1}H NMR ($CDCl_3$) δ 0.79 (6H, m), 1.16 (5H, m), 1.84 (1H, m), 2.23 (1H, dd, J = 16.6, 8.0), 2.43 (1H, dd, J = 16.6, 5.7), 3.31 (2H, s), 4.08 (2H, q, J = 7.1); ^{13}C NMR ($CDCl_3$) δ 11.0, 18.9, 29.1, 29.4, 30.2, 49.4, 49.8, 61.0, 166.9, 202.3. HRMS (ESI): for $C_{10}H_{18}O_3Na$ calcd: 209.1154 $[MNa]^+$; found: 209.1157.

5.29. Ethyl 6-methyl-3-oxoheptanoate (27e)

The title compound was prepared and purified according to the procedure described for **27b**, using 1-iodo-2-methylpropane instead of 1-iodobutane. The reaction yielded **27e** (51%), a colourless oil. Characterization data agreed with previous publication.[29]

5.30. Ethyl 6,6-dimethyl-3-oxoheptanoate (27f)

Oxalyl chloride (0.305 ml, 3.49 mmol) was added dropwise to a 0 °C solution of 4,4-dimethylpentanoic acid[30] (0.303 g, 2.33 mmol) and a catalytic amount of DMF in 5 ml CH_2Cl_2. The mixture was allowed to reach rt over 1 h and was then stirred for 2 h. The mixture was concentrated under reduced pressure and the resulting acid chloride was used, without further purification, as a reactant instead of valeroyl chloride according to the procedure described for **27a**. The crude product was purified by chromatography (petroleum ether/EtOAc, 30:1), yielding **27f** (0.208 g, 1.04 mmol, 45%), a colourless oil. ^{1}H NMR ($CDCl_3$) δ 0.89 (9H, s), 1.29 (3H, t, J = 7.1), 1.51 (2H, m), 2.52 (2H, m), 1.73 (2H, s), 4.21 (2H, q, J = 7.1); ^{13}C NMR ($CDCl_3$) δ 13.9, 28.9, 28.9, 28.9, 29.7, 36.7, 38.6, 49.2, 61.1, 167.1, 203.3. HRMS (ESI): for $C_{11}H_{21}O_3$ calcd: 201.1491 $[MH]^+$; found: 201.1483.

5.31. Ethyl 4-cyclopentyl-3-oxobutanoate (27g)

The title compound was prepared according to the procedure described for **27a**, using cyclopentylacetyl chloride instead of valeroyl chloride. The crude product was purified by chromatography (petroleum ether/EtOAc), yielding **27g** (64%), a colourless oil. Characterization data agreed with previous publication.[31]

5.32. Ethyl 4-cyclohexyl-3-oxobutanoate (27h)

The title compound was prepared and purified according to the same procedure described for **27f**, using phenylacetic acid instead of 4,4-dimethylpentanoic acid. The reaction yielded **27h** (78%), a colourless oil. ^{1}H NMR ($CDCl_3$) δ 0.94 (2H, m), 1.16 (2H, m), 1.28 (5H, m), 1.70 (4H, m), 1.86 (1H, m), 2.41 (2H, d, J = 6.8), 3.42 (2H, s), 4.21 (2H, q, J = 7.1); ^{13}C NMR ($CDCl_3$) δ 14.0, 25.9, 26.0, 26.0, 32.9, 32.9, 33.5, 49.7, 50.5, 61.1, 167.1, 202.3. HRMS (ESI): for $C_{12}H_{20}O_3Na$ calcd: 235.1310 $[MNa]^+$; found: 235.1317.

5.33. Ethyl 3-oxo-4-phenylbutanoate (27i)

The title compound was prepared and purified according to the procedure described for **27a**, using phenylacetyl chloride instead of valeroyl chloride. The reaction yielded **27i** (quantitative), a colourless oil. Characterization data agreed with previous publication.[15]

5.34. 1-Nitro-4-[(*E*)-2-phenylvinyl]benzene (28)

Styrene (0.698 ml, 6.02 mmol), 1-iodo-4-nitrobenzene (0.500 g, 2.01 mmol), K_2CO_3 (0.833 g, 6.02 mmol), tetrabutylammonium bromide (0.647 g, 2.01 mmol) and $Pd(OAc)_2$ (22 mg, 0.100 mmol) were mixed in 20 ml of DMF for 10 min, while flushing with N_2. The mixture was heated at 100 °C for 12 h, whereupon additionally styrene (0.698 ml, 6.02 mmol) was added and the mixture was stirred over night. Heating was removed and 25 ml of H_2O was added. The mixture was extracted with ether (3× 40 ml). The combined organic extract was washed with H_2O (40 ml) and dried with $MgSO_4$, yielding 4-nitrostilbene **28** (0.416 g, 1.85 mmol, 92%), characterization data agreed with previous publication.[32]

5.35. 2-(4-Nitrobenzyl)naphthalene (30)

$PdCl_2$ (6 mg, 0.0334 mmol) was added to a 0 °C suspension of 2-naphthaleneboronic acid (0.344 g, 2.00 mmol), 4-nitrobenzyl bromide (0.432 g, 2.00 mmol) and K_2CO_3 (0.692 g, 5.00 mmol) in acetone/H_2O (30:10 ml). The mixture was then allowed to reach rt and stirred for 3 h. The mixture was concentrated under reduced pressure, whereupon 25 ml H_2O was added. The mixture was extracted with diethyl ether (2× 40 ml), and the combined organic extract was washed with brine (40 ml) and dried with $MgSO_4$. The crude product was purified by chromatography (petroleum ether), yielding **30** (0.253 g, 9.61 mmol, 48%) as a brown solid (mp: 76–78 °C). ^{1}H NMR ($CDCl_3$) δ 4.25 (2H, s), 7.28 (1H, d, J = 6.8), 7.40 (2H, d, J = 8.5), 7.49 (2H, m), 7.65 (1H, s), 7.82 (3H, m), 8.17 (2H, d, J = 8.5); ^{13}C NMR ($CDCl_3$) δ 41.9, 123.8, 123.8, 124.0, 125.8, 126.3, 127.2, 127.4, 127.5, 127.7, 128.6, 129.7, 129.7, 132.3, 133.6, 136.6, 148.7. HRMS (EI): for $C_{17}H_{13}NO_2$ calcd: 263.0946 $[M]^+$; found: 263.0944.

5.36. 3-Benzylpyridine (32)

3-Benzoylpyridine (2.081 g, 11.36 mmol) and 10% Pd/C (0.8 g) was stirred in 100 ml of ethanol and 4 ml of concd HCl (aq) under H_2 at atmospheric pressure. After 24 h, the mixture was filtered and the filtrate was basified by 50 ml of 1 M NaOH (aq) followed by concentration under reduced pressure. The resulting mixture was extracted with diethyl ether (3× 50 ml). The combined organic extract was washed with 50 ml of brine and dried with $MgSO_4$, to give **32** (1.803 g, 10.65 mmol, 94%), a colourless oil. ^{1}H NMR ($CDCl_3$) δ 3.99 (2H, s), 7.26 (6H, m), 7.48 (1H, m), 8.47 (1H, d, J = 4.8), 8.53 (1H. m); ^{13}C NMR ($CDCl_3$) δ 38.4, 122.9, 125.9, 128.1, 128.1, 128.3, 128.3, 135.7, 136.0, 139.3, 147.0, 149.6. HRMS (ESI): for $C_{12}H_{12}N$ calcd: 170.0970 $[MH]^+$; found: 170.0970.

5.37. 3-(4-Nitrobenzyl)pyridine (33)

NH_4NO_3 (1.677 g, 20.954 mmol) was added in portions to a solution of **32** (1.773 g, 10.477 mmol), 10 ml trifluoroacetic anhydride and 15 ml $CHCl_3$ at −10 °C. The mixture was allowed to reach rt slowly while stirring under N_2. After 43 h, 150 ml of satd $NaHCO_3$ (aq) was added and the mixture was extracted with CH_2Cl_2 (3× 50 ml). The combined organic extract was washed with satd $NaHCO_3$ (aq) and dried with $MgSO_4$. The crudeproduct was purified by chromatography (heptane/ethyl acetate, 2:3), yielding **33** (1.233 g, 5.756 mmol, 55%) as a yellow solid (mp 87–88 °C). ^{1}H NMR ($CDCl_3$) δ 4.06 (2H, s), 7.22 (1H, dd, J = 7.8, 4.8), 7.31 (2H, d, J = 8.7), 7.46 (1H, m), 8.10 (2H, d, J = 8.7), 8.47 (2H, m); ^{13}C NMR ($CDCl_3$) δ 38.5, 123.6, 123.7, 123.7, 129.5, 129.5, 134.7, 136.4, 146.5, 147.2, 147.9, 149.7. HRMS (ESI): for $C_{12}H_{11}N_2O_2$ calcd: 215.0821 $[MH]^+$; found: 215.0810.

5.38. 4-(2-Phenylethyl)aniline (35)

28 (0.398 g, 1.77 mmol) and a catalytic amount of 10% Pd/C, in 25 ml MeOH was stirred over night under H_2, 500 psi. The mixture was filtered through Celite, yielding **35** (300 mg, 1.52 mmol, 86%), characterization data agreed with previous publication.[33]

5.39. 4-(3-Phenylpropyl)aniline (36)

Triphenyl(2-phenylethyl)phosphonium bromide was prepared as previously reported.[18] Butyllithium (1 ml 2.5 M soln in hexanes, 2.51 mmol) was added to a suspension of the phosphonium salt (1.07 g, 2.39 mmol) in 10 ml of THF at −78 °C, whereupon cooling was removed. After 20 min the mixture was cooled to −78 °C and 4-

nitrobenzaldehyde (0.361 g, 2.39 mmol) in 5 ml of THF was added dropwise. Cooling was removed and the mixture was stirred at rt for 90 min. Ten milliliters of brine was added and the mixture was extracted with ether (2× 25 ml). The combined organic extract was dried with $MgSO_4$. The crude product was purified by chromatography to give a mixture of the *E* and *Z* alkenes (**29**). The *E*/*Z* ratio was estimated to 75:25 on the basis of NMR data (identical with previous published data).[34] **29** and a catalytic amount of 10% Pd/C was stirred in 25 ml MeOH over night under H_2, 500 psi. The mixture was filtered through Celite, yielding **36** (0.338 g, 1.60 mmol, 67%) as a brown oil. ^{1}H NMR ($CDCl_3$) δ 1.99 (2H, quint, J = 7.6), 2.62 (2H, t, J = 7.6), 2.71 (2H, t, J = 7.6), 6.28 (2H, br s), 6.93 (2H, d, J = 7.8), 7.10 (2H, d, J = 7.8), 7.28 (3H, m), 7.36 (2H, d, J = 6.9); ^{13}C NMR ($CDCl_3$) δ 33.0, 34.5, 35.2, 117.4, 117.4, 125.6, 128.2, 128.2, 128.3, 128.3, 129.2, 129.2, 135.2, 139.4, 142.2. HRMS (ESI): for $C_{15}H_{18}N$ calcd: 212.1439 $[MH]^+$; found: 212.1429.

5.40. 4-(2-Naphthylmethyl)aniline (37)

2-(4-Nitrobenzyl)naphthalene (**30**) (0.247 g, 0.938 mmol) and a catalytic amount of 10% Pd/C in 20 ml EtOAc and 5 ml of EtOH was stirred over night under H_2, 500 psi. The mixture was filtered through Celite, washing with additionally EtOAc yielding **37** (218 mg, 0.938 mmol, quantitative), a brown solid (mp: 62–64 °C). ^{1}H NMR ($CDCl_3$) δ 4.16 (2H, s), 4.56 (2H, br s), 6.73 (2H, d, J = 8.3), 7.16 (2H, d, J = 8.1), 7.45 (1H, dd, J = 8.5, 1.6), 7.57 (2H, m), 7.75 (1H, s), 7.90 (3H, m); ^{13}C NMR ($CDCl_3$) δ 41.1, 115.4, 115.4, 125.1, 125.8, 126.7, 127.4, 127.5, 127.8, 129.7, 129.7, 131.0, 131.9, 133.5, 139.3, 144. HRMS (ESI): for $C_{17}H_{16}N$ calcd: 234.1283 $[MH]^+$; found: 234.1286.

5.41. 4-(Pyridin-4-ylmethyl)aniline (38)

4-(4-Nitrobenzyl)pyridine (**31**) (0.500 g, 2.33 mmol) and a catalytic amount of 10% Pd/C in 50 ml EtOH was stirred for 3 h under H_2 at atmospheric pressure. The mixture was filtered through Celite, washing with additionally EtOH yielding **38** (417 mg, 2.26 mmol, 97%). Characterization data agreed with previous publication.[35]

5.42. 4-(Pyridin-3-ylmethyl)aniline (39)

The title compound was prepared according to the procedure described for **38**, using **33** as reacting nitro compound. The reaction yielded **39** (133 mg, 0.722 mmol, 96%), a white solid (mp: 117–119 °C). ^{1}H NMR ($CDCl_3$) δ 3.84 (2H, s), 6.61 (2H, d, J = 8.1), 6.94 (2H, d, J = 8.1), 7.15 (1H, dd, J = 7.5, 4.7), 7.44 (1H, d, J = 7.5), 8.43 (1H, d, J = 4.7), 8.49 (1H, s); ^{13}C NMR ($CDCl_3$) δ 37.8, 115.0, 115.0, 123.1, 129.2, 129.4, 129.4, 135.9, 137.0, 144.8, 147.0, 149.7. HRMS (ESI): for $C_{12}H_{13}N_2$ calcd: 185.1079 $[MH]^+$; found: 185.1076.

5.43. 4-(Pyridin-2-ylmethyl)aniline (40)

2-(4-Nitrobenzyl)pyridine (**34**) was prepared from 2-benzylpyridine and used in the synthesis of **40**.[22]

5.44. Ethyl (2 *E*)-3-[(4-ethylphenyl)amino]-2-pentanoylacrylate (41)

27a (1.367 g, 7.94 mmol), triethyl orthoformate (3.97 ml, 23.81 mmol) and acetic anhydride (1.13 ml, 11.91 mmol) were mixed and heated at 100 °C, under nitrogen, over night. Low boiling components were evaporated under reduced pressure. The resulting crude product was mixed with 4-ethyl aniline (0.99ml, 7.94 mmol), whereupon the mixture was heated at 100 °C for 1 h. Low boiling components were evaporated under reduced pressure. The resulting crude product was purified by chromatography (petroleum ether/ethyl acetate, 30:1) yielding **41** (1.47 g, 4.84 mmol, 61%), a yellow oil. ^{1}H NMR ($CDCl_3$) δ 0.94 (3H, t, J = 7.3), 1.22 (3H, t, J = 7.6), 1.36 (5H, m), 1.62 (2H, quint, J = 7.5), 2.62 (2H, q, J = 7.6), 2.95 (2H, t, J = 7.5), 4.24 (2H, q, J = 7.1), 7.09 (2H, d, J = 8.3), 7.19 (2H, d, J = 8.3), 8.48 (1H, d, J = 13.2), 12.80 (1H, d, J = 13.2); ^{13}C NMR ($CDCl_3$) δ 14.0, 14.4, 15.4, 22.5, 27.0, 28.2, 41.9, 59.7, 101.9, 117.6, 117.6, 129.1, 129.1, 136.8, 141.7, 152.1, 166.9, 202.7. HRMS (ESI): for $C_{18}H_{26}NO_3$ calcd: 304.1913 $[MH]^+$; found: 304.1902.

5.45. Ethyl (2*E*)-3-[(4-ethylphenyl)amino]-2-hexanoylacrylate (42)

The title compound was prepared and purified according to the procedure described for **41**, starting from **27b** as reacting β-keto ester. The reaction yielded **42** (73%), a yellow oil. ^{1}H NMR ($CDCl_3$) δ 0.91 (3H, t, J = 6.7), 1.24 (3H, t, J = 7.6), 1.36 (7H, m), 1.66 (2H, m), 2.65 (2H, q, J = 7.6), 2.96 (2H, t, J = 7.6), 4.26 (2H, q, J = 7.1), 7.11 (2H, d, J = 8.5), 7.22 (2H, d, J = 8.5), 8.49 (1H, d, J = 13.2), 12.81 (1H, d, J = 13.2); ^{13}C NMR ($CDCl_3$) δ 14.0, 14.5, 15.5, 22.6, 24.7, 28.2, 31.7, 42.3, 59.8, 102.0, 117.8, 117.8, 129.2, 129.2, 136.9, 141.8, 152.2, 167.0, 202.9. HRMS (ESI): for $C_{19}H_{28}NO_3$ calcd: 318.2069 $[MH]^+$; found: 318.2056.

5.46. Ethyl (2*E*)-3-[(4-ethylphenyl)amino]-2-heptanoylacrylate (43)

The title compound was prepared and purified according to the procedure described for **41**, starting from **27c** as reacting β-keto ester. The reaction yielded **43** (55%), a yellow oil. ^{1}H NMR ($CDCl_3$) δ 0.88 (3H, t, J = 6.9), 1.22 (3H, t, J = 7.6), 1.34 (9H, m), 1.63 (2H, m), 2.62 (2H, q, J = 7.6), 2.95 (2H, t, J = 7.5), 4.24 (2H, q, J = 7.1), 7.09 (2H, d, J = 8.5), 7.19 (2H, d, J = 8.5), 8.48 (1H, d, J = 13.2), 12.80 (1H, d, J = 13.2); ^{13}C NMR ($CDCl_3$) δ 14.0, 14.4, 15.4, 22.5, 24.8, 28.1, 29.2, 31.7, 42.2, 59.7, 101.9, 117.6, 117.6, 129.1, 129.1, 136.8, 141.6, 152.1, 166.8, 202.7. HRMS (ESI): for $C_{20}H_{29}NO_3Na$ calcd: 354.2045 $[MNa]^+$; found: 354.2035.

5.47. Ethyl (2*E*)-3-[(4-ethylphenyl)amino]-2-(3-methylpentanoyl) acrylate (44)

The title compound was prepared and purified according to the procedure described for **41**, starting from **27d** as reacting β-keto ester. The reaction yielded **44** (78%), a yellow oil. ^{1}H NMR ($CDCl_3$) δ 0.91 (6H, m), 1.30 (8H, m), 1.99 (1H, m), 2.63 (2H, q, J = 7.6), 2.78 (1H, dd, J = 15.3, 7.9), 2.97 (1H, dd, J = 15.3, 5.8), 4.20 (2H, q, J = 7.1), 7.10 (2H, d, J = 8.4), 7.21 (2H, d, J = 8.4), 8.48 (1H, d, J = 13.2), 12.84 (1H, d, J = 13.2); ^{13}C NMR ($CDCl_3$) δ 11.4, 14.5, 15.5, 19.4, 28.2, 29.7, 31.5, 49.1, 59.8, 102.4, 117.7, 117.7, 129.1, 129.1, 136.9, 141.7, 152.2, 167.0, 202.5. HRMS (ESI): for $C_{19}H_{28}NO_3$ calcd: 318.2069 $[MH]^+$; found: 318.2063.

5.48. Ethyl (2*E*)-3-[(4-ethylphenyl)amino]-2-(4-methylpentanoyl) acrylate (45)

The title compound was prepared and purified according to the procedure described for **41**, starting from **27e** as reacting β-keto ester. The reaction yielded **45** (81%), a yellow oil. ^{1}H NMR ($CDCl_3$) δ 0.94 (6H, d, J = 6.6), 1.22 (3H, t, J = 7.6), 1.34 (3H, t, J = 7.1), 1.53 (2H, m), 1.62 (1H, m), 2.63 (2H, q, J = 7.6), 2.97 (2H, t, J = 7.8), 4.25 (2H, q, J = 7.1), 7.09 (2H, d, J = 8.4), 7.20 (2H, d, J = 8.4), 8.48 (1H, d, J = 13.2), 12.81 (1H, d, J = 13.2); ^{13}C NMR ($CDCl_3$) δ 14.5, 15.4, 22.5, 22.5, 27.9, 28.2, 33.9, 40.3, 59.8, 102.0, 117.7, 117.7, 129.1, 129.1, 136.9, 141.7, 152.2, 166.9, 203.0. HRMS (ESI): for $C_{19}H_{28}NO_3$ calcd: 318.2069 $[MH]^+$; found: 318.2054.

5.49. Ethyl (2*E*)-2-(4,4-dimethylpentanoyl)-3-[(4-ethylphenyl) amino]acrylate (46)

The title compound was prepared according to the procedure described for **41**, starting from **27f** as reacting β-keto ester. The final crude product was purified by chromatography (petroleum ether/ethyl acetate, 30:1) followed by recrystallization from petroleum ether at −18 °C, yielding **46** (21%), a colourless solid (mp: 71–73 °C). ^{1}H NMR ($CDCl_3$) δ 0.95 (9H, s), 1.23 (3H, t, J = 7.6), 1.35 (3H, t, J = 7.1), 1.55 (2H, m), 2.64 (2H, q, J = 7.6), 2.95 (2H, m), 4.26 (2H, q, J = 7.1), 7.09 (2H, d, J = 8.4), 7.21 (2H, d, J = 8.4), 8.49 (1H, d, J = 1.2), 12.81 (1H, d, J = 13.2); ^{13}C NMR ($CDCl_3$) δ 14.5, 15.5, 28.2, 29.3, 29.3, 29.3, 30.1, 37.9, 38.6, 59.8, 102.0, 117.7, 117.7, 129.1, 129.1, 136.9, 141.7, 152.2, 166.9, 203.3. HRMS (ESI): for $C_{20}H_{29}NO_3$-Na calcd: 354.2045 $[MNa]^+$; found: 354.2031.

5.50. Ethyl (2*E*)-2-(cyclopentylacetyl)-3-[(4-ethylphenyl) amino]acrylate (47)

The title compound was prepared and purified according to the procedure described for **41**, starting from **27g** as reacting β-keto ester. The reaction yielded **47** (76%), a yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.19 (2H, m), 1.24 (3H, t, J = 7.6), 1.35 (3H, t, J = 7.1), 1.59 (4H, m), 1.84 (2H, m), 2.32 (1H, m), 2.65 (2H, q, J = 7.6), 3.00 (2H, d, J = 7.1), 4.26 (2H, q, J = 7.1), 7.11 (2H, d, J = 8.3), 7.22 (2H, d, J = 8.3), 8.49 (1H, d, J = 13.2), 12.82 (1H, d, J = 13.2); ^{13}C NMR ($CDCl_3$) δ 14.5, 15.5, 25.0, 25.0, 28.2, 32.7, 32.7, 36.3, 48.3, 59.8, 102.2, 117.8, 117.8, 129.2, 129.2, 137.0, 141.8, 152.2, 167.0, 202.6. HRMS (ESI): for $C_{20}H_{28}NO_3$ calcd: 330.2069 $[MH]^+$; found: 330.2060.

5.51. Ethyl (2*E*)-2-(cyclohexylacetyl)-3-[(4-ethylphenyl)amino]-acrylate (48)

The title compound was prepared and purified according to the procedure described for **41**, starting from **27h** as reacting β-keto ester. The reaction yielded **48** (62%), a yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.02 (2H, m), 1.17 (2H, m), 1.24 (3H, t, J = 7.5), 1.29 (2H, m), 1.35 (3H, t, J = 7.1), 1.70 (4H, m), 1.88 (1H, m), 2.65 (2H, q, J = 7.5), 2.85 (2H, d, J = 6.7), 4.26 (2H, q, J = 7.1), 7.11 (2H, d, J = 8.3), 7.22 (2H, d, J = 8.3), 8.49 (1H, d, J = 13.2), 12.85 (1H, d, J = 13.2); ^{13}C NMR ($CDCl_3$) δ 15.0, 15.5, 26.3, 26.3, 26.4, 28.2, 33.4, 33.4, 34.9, 49.6, 59.9, 102.5, 117.8, 117.8, 129.2, 129.2, 136.9, 141.8, 152.3, 167.0, 202.3. HRMS (ESI): for $C_{21}H_{30}NO_3$ calcd: 344.2226 $[MH]^+$; found: 344.2222.

5.52. Ethyl (2*E*)-3-[(4-ethylphenyl)amino]-2-(phenylacetyl)-acrylate (49)

The title compound was prepared and purified according to the procedure described for **41**, starting from **27i** as reacting β-keto ester. The reaction yielded **49** (46%), a yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.26 (3H, t, J = 7.6), 1.39 (3H, t, J = 7.1), 2.67 (2H, q, J = 7.6), 4.32 (2H, q, J = 7.1), 4.38 (2H, s), 7.11 (2H, d, J = 8.5), 7.31 (7H, m), 8.56 (1H, d, J = 13.3), 12.77 (1H, d, J = 13.3); ^{13}C NMR ($CDCl_3$) δ 14.5, 15.5, 28.2, 48.3, 60.0, 101.7, 117.8, 117.8, 126.4, 128.3, 128.3, 129.2, 129.2, 129.8, 129.8, 136.7, 142.0, 152.7, 166.9, 199.5. HRMS (ESI): for $C_{21}H_{23}NO_3Na$ calcd: 360.1576 $[MNa]^+$; found: 360.1586.

5.53. Ethyl (2*E*)-3-anilino-2-pentanoylacrylate (50)

The title compound was prepared and purified according to the procedure described for **41**, but using aniline instead of 4-ethylaniline. The reaction yielded **50** (68%), a yellow oil. ^{1}H NMR ($CDCl_3$) δ 0.94 (3H, t, J = 7.3), 1.34 (3H, t, J = 7.1), 1.39 (2H, m), 1.63 (2H, quint, J = 7.5), 2.96 (2H, t, J = 7.5), 4.26 (2H, q, J = 7.1), 7.17 (3H, m), 7.38 (2H, t, J = 7.8), 8.50 (1H, d, J = 13.1), 12.79 (1H, d, J = 13.1); ^{13}C NMR ($CDCl_3$) δ 14.0, 14.4, 22.5, 27.0, 42.0, 59.8, 102.4, 117.6, 117.6, 125.4, 129.8, 129.8, 139.1, 151.9, 166.8, 202.9. HRMS (ESI): for $C_{16}H_{22}NO_3$ calcd: 276.1600 $[MH]^+$; found: 276.1590.

5.54. Ethyl (2*E*)-3-[(4-bromophenyl)amino]-2-pentanoylacrylate (51)

The title compound was prepared and purified according to the procedure described for **41**, but using 4-bromoaniline instead of 4-ethylaniline. The reaction yielded **51** (59%), a yellow oil. ^{1}H NMR ($CDCl_3$) δ 0.90 (3H, t, J = 7.2), 1.31 (3H, t, J = 6.9), 1.35 (2H, m), 1.57 (2H, quint, J = 7.4), 2.91 (2H, t, J = 7.4), 4.22 (2H, q, J = 6.9), 7.00 (2H, d, J = 8.2), 7.43 (2H, d, J = 8.2), 8.38 (1H, d, J = 12.7), 12.74 (1H, d, J = 12.7); ^{13}C NMR ($CDCl_3$) δ 13.9, 14.3, 22.4, 26.8, 42.0, 59.9, 102.8, 118.0, 118.9, 118.9, 132.7, 132.7, 138.1, 151.3, 166.5, 202.9. HRMS (ESI): for $C_{16}H_{21}NO_3Br$ calcd: 354.0705 $[MH]^+$; found: 354.0692.

5.55. Ethyl (2*E*)-3-(1,1′-biphenyl-4-ylamino)-2-pentanoylacrylate (52)

The title compound was prepared and purified according to the procedure described for **41**, but using 4-phenylaniline instead of 4-ethylaniline. The reaction yielded **52** (65%), a yellow oil. ^{1}H NMR ($CDCl_3$) δ 0.96 (3H, t, J = 7.3), 1.37 (3H, t, J = 7.1), 1.40 (2H, m), 1.64 (2H, m), 2.99 (2H, t, J = 7.5), 4.29 (2H, q, J = 7.1), 7.27 (2H, d, J = 8.7), 7.37 (1H, m), 7.46 (2H, m), 7.61 (4H, m), 8.56 (1H, d, J = 13.1), 12.89 (1H, d, J = 13.1); ^{13}C NMR ($CDCl_3$) δ 14.1, 14.5, 22.6, 27.1, 42.1, 60.0, 102.6, 118.0, 118.0, 126.8, 126.8, 127.5, 128.4, 128.4, 128.9, 128.9, 138.3, 138.4, 139.9, 151.7, 166.9, 203.1. HRMS (ESI): for $C_{22}H_{26}NO_3$ calcd: 352.1913 $[MH]^+$; found: 352.1899.

5.56. Ethyl (2*E*)-3-[(4-benzylphenyl)amino]-2-pentanoylacrylate (53)

The title compound was prepared and purified according to the procedure described for **41**, but using 4-benzylaniline instead of 4-ethylaniline. The reaction yielded **53** (68%), a yellow oil. ^{1}H NMR ($CDCl_3$) δ 0.97 (3H, t, J = 7.2), 1.36 (3H, t, J = 7.1), 1.40 (2H, m), 1.65 (2H, quint, J = 7.4), 2.99 (2H, t, J = 7.4), 3.99 (2H, s), 4.27 (2H, q, J = 7.1), 7.12 (2H, d, J = 8.3), 7.22 (5H, m), 7.32 (2H, d, J = 7.4), 8.50 (1H, d, J = 13.2), 12.82 (1H, d, J = 13.2); ^{13}C NMR ($CDCl_3$) δ 14.0, 14.4, 22.5, 27.0, 41.2, 42.0, 59.8, 102.1, 117.8, 117.8, 126.2, 128.5, 128.5, 128.8, 128.8, 130.2, 130.2, 137.3, 138.6, 140.5, 152.0, 166.8, 202.8. HRMS (ESI): for $C_{23}H_{28}NO_3$ calcd: 366.2069 $[MH]^+$; found: 366.2056.

5.57. Ethyl (2*E*)-3-[(4-benzylphenyl)amino]-2-butyrylacrylate (54)

The title compound was prepared and purified according to the procedure described for **41**, starting from ethyl 3-oxohexanoate as reacting β-keto ester and using 4-benzylaniline instead of 4-ethylaniline. The reaction yielded **54** (79%), a yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.01 (3H, t, J = 7.4), 1.35 (3H, t, J = 7.1), 1.70 (2H, sex, J = 7.4), 2.97 (2H, t, J = 7.4), 3.97 (2H, s), 4.27 (2H, q, J = 7.1), 7.11 (2H, d, J = 7.1), 7.21 (5H, m), 7.30 (2H. m), 8.49 (1H, d, J = 13.2), 12.82 (1H, d, J = 13.2); ^{13}C NMR ($CDCl_3$) δ 13.9, 14.4, 18.1, 41.1, 44.1, 59.7, 102.1, 117.7, 117.7, 126.1, 128.4, 128.4, 128.7, 128.7, 130.1, 130.1, 137.2, 138.5, 140.5, 151.9, 166.7, 202.6. HRMS (ESI): for $C_{22}H_{26}NO_3$ calcd: 352.1913 $[MH]^+$; found: 352.1899.

5.58. Ethyl (2*E*)-3-[(4-benzylphenyl)amino]-2-(cyclopentylacetyl) acrylate (55)

The title compound was prepared and purified according to the procedure described for **41**, starting from **27g** as reacting β-keto ester and using 4-benzylaniline instead of 4-ethylaniline. The reaction yielded **55** (72%), a yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.22 (2H, m), 1.34 (3H, t, J = 7.1), 1.55 (2H, m), 1.63 (2H, m), 1.84 (2H, m), 2.33 (1H, m), 3.01 (2H, d, J = 7.1), 3.97 (2H, s), 4.26 (2H, q, J = 7.1), 7.11 (2H, d, J = 8.4), 7.18 (3H, m), 7.22 (2H, d, J = 7.5), 7.30 (2H, t, J = 7.5), 8.48 (1H, d, J = 13.1), 12.83 (1H, d, J = 13.1); ^{13}C NMR ($CDCl_3$) δ 14.4, 24.9, 24.9, 32.6, 32.6, 36.2, 41.1, 48.2, 59.7, 102.3, 117.7, 117.7, 126.2, 128.5, 128.5, 128.7, 128.7, 130.1, 130.1, 137.3, 138.5, 140.5, 151.9, 166.8, 202.5. HRMS (ESI): for $C_{25}H_{30}NO_3$ calcd: 392.2226 $[MH]^+$; found: 392.2222.

5.59. Ethyl (2*E*)-2-pentanoyl-3-{[4-(2-phenylethyl)phenyl]-amino}acrylate (56)

The title compound was prepared and purified according to the procedure described for **41**, but using **35** instead of 4-ethylaniline. The reaction yielded **56** (46%), a yellow oil. ^{1}H NMR ($CDCl_3$) δ 0.99 (3H, t, J = 7.3), 1.39 (3H, t, J = 7.1), 1.43 (2H, m), 1.67 (2H, quint, J = 7.5), 2.96 (4H, s), 3.01 (2H, t, J = 7.5), 4.30 (2H, q, J = 7.1), 7.13 (2H, d, J = 8.5), 7.22 (5H, m), 7.32 (2H, d, J = 7.1), 8.53 (1H, d, J = 13.2), 12.84 (1H, d, J = 13.2); ^{13}C NMR ($CDCl_3$) δ 14.0, 14.4, 22.6, 27.0, 37.1, 37.7, 42.0, 59.8, 102.0, 117.6, 117.6, 125.9, 128.3, 128.3, 128.4, 128.4, 129.8, 129.8, 137.1, 139.1, 141.1, 152.0, 166.9, 202.8. HRMS (ESI): for $C_{24}H_{30}NO_3$ calcd: 380.2226 $[MH]^+$; found: 380.2222.

5.60. Ethyl (2*E*)-2-pentanoyl-3-{[4-(3-phenylpropyl)phenyl]-amino} acrylate (57)

The title compound was prepared and purified according to the procedure described for **41**, but using **36** instead of 4-ethylaniline. The reaction yielded **57** (60%), a yellow oil. ^{1}H NMR ($CDCl_3$) δ 0.96 (3H, t, J = 7.3), 1.35 (3H, t, J = 7.1), 1.40 (2H, m), 1.64 (2H, m), 1.95 (2H, m), 2.65 (4H, m), 2.98 (2H, t, J = 7.5), 4.27 (2H, q, J = 7.1), 7.11 (2H, d, J = 8.5), 7.20 (5H, m), 7.30 (2H, m), 8.50 (1H, d, J = 13.2), 12.83 (1H, d, J = 13.2); ^{13}C NMR ($CDCl_3$) δ 14.0, 14.5, 22.6, 27.1, 32.8, 34.7, 35.3, 42.0, 59.8, 102.0, 117.7, 117.7, 125.8, 128.3, 128.3, 128.3, 128.3, 129.7, 129.7, 137.0, 139.8, 141.9, 152.1, 166.9, 202.8. HRMS (ESI): for $C_{25}H_{32}NO_3$ calcd: 394.2382 $[MH]^+$; found: 394.2384.

5.61. Ethyl (2*E*)-3-{[4-(2-naphthylmethyl)phenyl]amino}-2-pentanoylacrylate (58)

The title compound was prepared and purified according to the procedure described for **41**, but using **37** instead of 4-ethylaniline. The reaction yielded **58** (68%), a yellow oil. ^{1}H NMR ($CDCl_3$) δ 0.99 (3H, t, J = 7.3), 1.36 (3H, t, J = 7.1), 1.43 (2H, m), 1.67 (2H, quint, J = 7.5), 3.01 (2H, t, J = 7.5), 4.13 (2H, s), 4.28 (2H, q, J = 7.1), 7.12 (2H, d, J = 8.4), 7.25 (2H, d, J = 8.4), 7.31 (1H, dd, J = 8.4, 1.6), 7.47 (2H, m), 7.63 (1H, br s), 7.81 (3H, m), 8.51 (1H, d, J = 13.2), 12.85 (1H, d, J = 13.2); ^{13}C NMR ($CDCl_3$) δ 14.0, 14.4, 22.5, 27.0, 41.3, 42.0, 59.8, 102.1, 117.7, 117.7, 125.4, 126.0, 127.0, 127.3, 127.4, 127.5, 128.1, 130.2, 130.2, 132.0, 133.5, 137.3, 138.0, 138.4, 151.9, 166.8, 202.8. HRMS (ESI): for $C_{27}H_{30}NO_3$ calcd: 416.2226 $[MH]^+$; found: 416.2225.

5.62. Ethyl (2*E*)-2-pentanoyl-3-{[4-(pyridin-4-ylmethyl) phenyl]amino}acrylate (59)

The title compound was prepared and purified according to the procedure described for **41**, but using **38** instead of 4-ethylaniline. The reaction yielded **59** (46%), a yellow oil. ^{1}H NMR ($CDCl_3$) δ 0.94 (3H, t, J = 7.6), 1.34 (3H, t, J = 7.1), 1.38 (2H, m), 1.62 (2H, quint, J = 7.5), 2.96 (2H, t, J = 7.5), 3.96 (2H, s), 4.25 (2H, q, J = 7.1), 7.15 (6H, m), 8.49 (3H, m), 12.80 (1H, d, J = 13.0); ^{13}C NMR ($CDCl_3$) δ 14.0, 14.4, 25.6, 27.0, 40.5, 42.0, 59.9, 102.4, 117.6, 118.0, 118.0, 124.1, 124.1, 130.4, 130.4, 136.1, 137.9, 149.7, 149.7, 151.9, 166.8, 203.0. HRMS (ESI): for $C_{22}H_{27}N_2O_3$ calcd: 367.2022 $[MH]^+$; found: 367.2015.

5.63. Ethyl (2*E*)-2-pentanoyl-3-{[4-(pyridin-3-ylmethyl)phenyl]-amino} acrylate (60)

The title compound was prepared and purified according to the procedure described for **41**, but using **39** instead of 4-ethylaniline. The reaction yielded **60** (48%), a yellow oil. ^{1}H NMR ($CDCl_3$) δ 0.90 (3H, t, J = 7.3), 1.30 (3H, t, J = 7.1), 1.35 (2H, m), 1.59 (2H, quint, J = 7.5), 2.93 (2H, t, J = 7.5), 3.93 (2H, s), 4.22 (2H, q, J = 7.1), 7.09 (2H, d, J = 8.5), 7.18 (3H, m), 7.43 (1H, m), 8.44 (3H, m), 12.77 (1H, d, J = 13.2); ^{13}C NMR ($CDCl_3$) δ 13.9, 14.3, 22.5, 26.9, 38.2, 41.9, 59.8, 102.3, 117.8, 117.8, 123.4, 130.1, 130.1, 135.9, 136.1, 137.0, 137.6, 147.6, 149.9, 151.8, 166.7, 202.8. HRMS (ESI): for $C_{22}H_{27}N_2O_3$ calcd: 367.2022 $[MH]^+$; found: 367.2017.

5.64. Ethyl (2*E*)-2-pentanoyl-3-{[4-(pyridin-2-ylmethyl)-phenyl]amino} acrylate (61)

The title compound was prepared and purified according to the procedure described for **41**, but using **40**[22] instead of 4-ethylaniline. The reaction yielded **61** (51%), a yellow oil. ^{1}H NMR ($CDCl_3$) δ 0.88 (3H, t, J = 7.3), 1.27 (3H, t, J = 7.1), 1.33 (2H, m), 1.57 (2H, quint, J = 7.5), 2.90 (2H, t, J = 7.5), 4.07 (2H, s), 4.19 (2H, q, J = 7.1), 7.05 (4H, m), 7.22 (2H, d, J = 8.3), 7.52 (1H, dt, J = 7.7, 1.5), 8.42 (1H, d, J = 13.1), 8.48 (1H, m), 12.74 (1H, d, J = 13.1); ^{13}C NMR ($CDCl_3$) δ 13.8, 14.3, 22.4, 26.8, 41.8, 43.7, 59.6, 102.0, 117.7, 117.7, 121.2, 122.8, 130.2, 130.2, 136.4, 136.7, 137.3, 149.2, 151.8, 160.2, 166.6, 202.6. HRMS (ESI): for $C_{22}H_{27}N_2O_3$ calcd: 367.2022 $[MH]^+$; found: 367.2015.

5.65. Ethyl (2*E*)-2-(cyclopentylacetyl)-3-{[4-(pyridin-3-ylmethyl) phenyl]amino}acrylate (62)

The title compound was prepared and purified according to the procedure described for **41**, starting from **27g** as reacting β-keto ester and using **39** instead of 4-ethylaniline. The reaction yielded **62** (38%), a yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.14 (2H, m), 1.29 (3H, t, J = 7.1), 1.53 (4H, m), 1.78 (2H, m), 2.26 (1H, m), 2.95 (2H, d, J = 7.1), 3.92 (2H, s), 4.20 (2H, q, J = 7.1), 7.08 (2H. d. J = 8.5), 7.16 (3H, m), 7.41 (1H, d, J = 7.8), 8.43 (3H, m), 12.8 (1H, d, J = 13.2); ^{13}C NMR ($CDCl_3$) δ 14.3, 24.8, 24.8, 32.5, 32.5, 36.1, 38.2, 48.1, 59.7, 102.4, 117.8, 117.8, 123.3, 130.0, 130.0, 135.9, 136.1, 136.9, 137.6, 147.6, 149.8, 151.7, 166.7, 202.5. HRMS (ESI): for $C_{24}H_{29}N_2O_3$ calcd: 393.2178 $[MH]^+$; found: 393.2166.

5.66. Ethyl (2*E*)-3-amino-2-pentanoylacrylate (63)

The acyl-ethoxy acrylate-intermediate was prepared as described in the synthesis of **41**. This crude intermediate was stirred at rt in 5 ml of 2.0 M NH_3 solution in EtOH for 5 h. Solvent was evaporated under reduced pressure and the crude product was purified by chromatography (petroleum ether/ethyl acetate, 6:1) yielding **63** (42%), a yellow oil. ^{1}H NMR ($CDCl_3$) δ 0.93 (3H, t, J = 7.3), 1.30 (3H, t, J = 7.1), 1.37 (2H, m), 1.59 (2H, quint, J = 7.6), 2.89 (2H, t, J = 7.6), 4.20 (2H, q, J = 7.1), 5.91 (1H, br s), 8.11 (1H, dd, J = 15.1, 8.7), 10.31 (1H, br s); ^{13}C NMR ($CDCl_3$) δ 14.0, 14.4, 22.6, 27.1, 42.2, 59.6, 101.8, 158.1, 167.2, 203.2. HRMS (ESI): for $C_{10}H_{18}NO_3$ calcd: 200.1287 $[MH]^+$; found: 200.1289.

5.67. Ethyl (2*E*)-3-(cyclohex-1-en-1-ylamino)-2-pentanoylacrylate (64)

63 (145 mg, 0.728 mmol), cyclohexanone (107 mg, 1.09 mmol) and a catalytic amount of *p*-TsOH were refluxed in 10 ml of toluene under Dean-Stark conditions for 24 h. The mixture was neutralized by a small amount of Na_2CO_3, followed by concentration under reduced pressure. The resulting crude product was purified by chromatography (petroleum ether/EtOAc, 9:1) yielding **64** (97 mg, 0.347 mmol, 48%), a yellow oil. ^{1}H NMR ($CDCl_3$) δ 0.88 (3H, t, J = 7.4), 1.27 (3H, t, J = 7.1), 1.33 (2H, pent, J = 7.4), 1.55 (4H, m), 1.73 (2H, m), 2.10 (2H, m), 2.17 (2H, m), 2.86 (2H, t, J = 7.6), 4.17 (2H, q, J = 7.1), 5.47 (1H, t, J = 4.1), 8.07 (1H, d, J = 13.5), 12.49 (1H, d, J = 13.5); ^{13}C NMR ($CDCl_3$) δ 13.9, 14.4, 21.8, 21.8, 22.5, 24.0, 24.0, 27.2, 41.8, 59.4, 100.2, 113.0, 135.0, 150.4, 167.1, 202.1. HRMS (ESI): for $C_{16}H_{26}NO_3$ calcd: 280.1913 $[MH]^+$; found: 280.1899.

5.68. Benzodiazepine receptor binding in vitro

Binding of ^{3}H-flumazenil (87 Ci/mmol) to rat cortical membranes and to a membrane suspension of HEK 293 cells expressing human $\alpha_1\beta_2\gamma_{2s}$ $\alpha_2\beta_2\gamma_{2s}$ or $\alpha_3\beta_2\gamma_{2s}$ $GABA_A$ receptors was done following methods previously described by Kahnberg et al.[11] In brief: tissue is homogenized in 20 ml Tris, HCl (30 mM, pH 7.4) using an Ultra-Turrax homogenizer. The suspensions are centrifuged at 27,000g for 15 min followed by three centrifugations resuspensions cycles. The washed pellet is resuspended in 20 ml buffer, incubated at 37 °C for 30 min and then centrifuged for 10 min (27,000*g*). The pellet is washed once and the final pellet is resuspended in 30 ml Tris–HCl buffer (50 mM, pH 7.1) and stored at −20 °C until use. For binding studies, frozen membrane suspensions were thawed and centrifuged (27,000*g*, 10 min). The pellet was resuspended into Tris,citrate buffer (50 mM, pH 7.1) at a tissue concentration: cortex preparation ca. 50 μg protein/0.55 ml assay (1 mg original tissue/0.55 ml assay) and HEK cells ca 25 μg protein per 0.55 ml assay. Aliquots of 0.5 ml membrane preparation are added to 25 μl of ^{3}H-flumazenil solution (1 nM final concentration) and 25 μl containing test substance and incubated at an ice-bath (0–4 °C) for 40 min. The incubated samples were added 5 ml ice-cold buffer (Tris, citrate, 50 mM pH 7.1), poured direcly onto Whatman GF/C glass fibre filters under suction and immediately washed with 5 ml ice-cold buffer. Non-specific binding was determined by adding Clonazepam (1 μM final concentration) to separate samples. Protein was estimated by conventional protein assay method using Bovine serum albumin as standard.

IC_{50} values were determined using 4–6 different concentrations of test substance. K_i values were calculated according to $K_i = IC_{50}/(1 + (L)/K_D)$, (L) is the concentration (nM) of ^{3}H-flumazenil; K_D is binding affinity constant of ^{3}H-flumazenil (1.6 nM).

5.69. Computational methods

Conformational analyses were performed by using the Monte Carlo multiple minimum (MCMM) method[36] with default setting as implemented in MacroModel 7.0.[37] Force field calculations were carried out using the MMFF94s force field[38] with solvation effects calculated by the GB/SA hydration model.[39] The energy minimizations were carried out using the Polak-Ribiere conjugate gradient algorithm (PRCG) as implemented in MacroModel 7.0.

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