Bioorganic Chemistry 40 (2012) 125–130

Contents lists available at SciVerse ScienceDirect

Bioorganic Chemistry

journal homepage: www.elsevier.com/locate/bioorg

3-Alkyl- and 3-amido-isothiazoloquinolin-4-ones as ligands for the benzodiazepine site of $GABA_A$ receptors

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ARTICLE INFO

Article history:
Received 13 September 2011
Available online 18 October 2011

Keywords:
3-Alkyl-6-methylisothiazolo[5,4-*b*]quinolin-4(9*H*)-ones
3-Amido-6-methylisothiazolo[5,4-*b*]quinolin-4(9*H*)-ones
Benzodiazepine binding site
$GABA_A$ receptors
Pharmacophore model

ABSTRACT

Based on a pharmacophore model of the benzodiazepine binding site of the $GABA_A$ receptors, developed with synthetic flavones and potent 3-carbonylquinolin-4-ones, 3-alkyl- and 3-amido-6-methylisothiazoloquinolin-4-ones were designed, prepared and assayed. The suggestion that the interaction between the hydrogen bond donor site H1 with the 3-carbonyl oxygen in 3-carbonylquinolin-4-ones can be replaced by an interaction between H1 and N-2 in the isothiazoloquinolin-4-ones, was confirmed. As with the 3-carbonylquinolin-4-ones, the length of the chain in position 3 is critical for an efficient interaction with the lipophilic pockets of the pharmacophore model. The most potent 3-alkyl derivative, 3-pentyl-6-methylisothiazoloquinolin-4-one, has an affinity (K_i value) for the benzodiazepine binding site of the $GABA_A$ receptors of 13 nM. However, by replacing the 3-pentyl with a 3-butyramido group an even more potent compound was obtained, with a K_i value of 2.8 nM, indicating that the amide function facilitates additional interactions with the binding site.

1. Introduction

The major inhibitory neurotransmitter in the central nervous system is γ-aminobutyric acid (GABA) [1]. Ionotropic receptors for GABA are ligand gated ion channels that on activation by GABA mediate fast neurotransmission by allowing a flow of chloride ions into the neuron, causing a hyperpolarization of the membrane and inhibiting further neuronal activity. Allosteric modulatory sites on $GABA_A$ receptors exist for various ligands including benzodiazepine, picrotoxin, loreclezole, ethanol, barbiturate and zinc cations, of which the benzodiazepine site has attracted most attention.

Full agonists acting at the benzodiazepine site have long been used as anxiolytics, although their applicability is limited due to adverse effects such as sedation, cognitive impairment and ataxia. An earlier pharmacophore model of the benzodiazepine binding site [2] has been developed and refined based on SAR studies of synthetic flavone derivatives [3,4]. The model was recently applied for the identification and optimization of novel 3-carbonylquinolin-4-ones [5,6], azaflavones [7] and triazoloquinazolinediones [8] as ligands at the $GABA_A$ receptors. Fig. 1 shows the structures of three 3-carbonylquinolin-4-one derivatives that bind strongly to the benzodiazepine binding site of the $GABA_A$ receptors, 3-valeryl-6-methylquinolin-4-one **1**, 3-propyloxycarbonyl-6-methylquinolin-4-one **2** and 3-(*N*-propylcarbamoyl)-6-methylquinolin-4-one **3**. The affinity (expressed as the K_i value) of **1**, **2** as well as **3** for the benzodiazepine binding site is approximately 1 nM [5,6].

Fig. 2 shows the present pharmacophore model (left), and compound **1** positioned in the pharmacophore model (right). The 3-carbonyl oxygen of **1** is believed to interact with the hydrogen bond donating site H1 in the pharmacophore model, and in a search for new scaffolds for binding site we believed that this interaction can be replaced with that between H1 and N-2 in suitably substituted isothiazoloquinolin-4-ones. As can be seen in the pharmacophore model (Fig. 2, left) there is little room for substituents, essentially only the lipophilic pocket towards L2 and the interface region (an area between an α and a γ subunit in the pentaheteromeric receptor $GABA_A$). In this study we have focussed on the lipophilic pocket and kept the substituent in position 6 constant. Consequently, compounds **13**, **14** and **15** were designed, synthesized, and assayed. As the presence of a carbamoyl function at position 3 in the quinolin-4-ones (e.g. **3**, Fig. 1) in some cases have been shown to be advantageous, we decided to include compound **18** and **19** as well (having an amido group instead of a carbamoyl group in position 3).

2. Material and methods

2.1. General procedures for synthesis

Reagents and solvents (except THF) were used from commercial sources without purification. THF was distilled from sodium/

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doi:10.1016/j.bioorg.2011.10.001

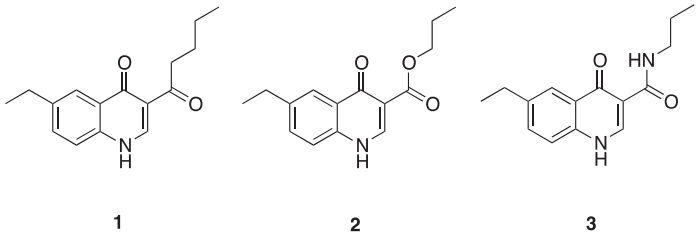


Fig. 1. Potent 3-carbonyl-6-ethylisothiazoloquinolinones representing the starting point for this study.

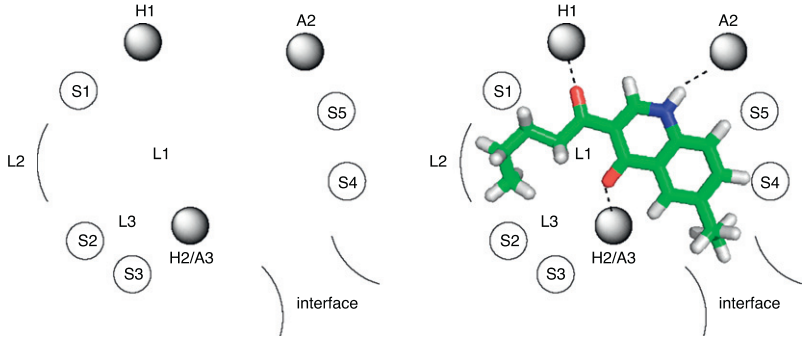


Fig. 2. Left: The pharmacophore model used for the design of the new structures. H1 and H2 are hydrogen bond donor sites while A2 and A3 are hydrogen bond acceptor sites. L1, L2 and L3 represent lipophilic pockets and S1–S5 denotes regions of steric repulsive ligand-receptor interactions (receptor essential volume). The interface region is a partly lipophilic region that has been suggested to represent the interface between α- and γ-subunits in $GABA_A$ receptors. Right: 3-valeryl-6-ethylisothiazoloquinolinone (**1**) positioned in the model.

benzophenone prior to use. ^1H and ^{13}C NMR were recorded at room temperature unless otherwise specified with a Bruker DR400 spectrometer. The spectra were recorded in $CDCl_3$, DMSO-d_6, and C_6D_6, and the solvent signals (7.27 and 77.0, 2.50 and 39.5 or 7.18 and 128.1 ppm, respectively) were used as reference. 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.

*2.1.1. 5-Methyl-2-{[(methylsulfanyl)carbonothioyl]amino}benzoic acid **(5)***

To a solution of 2-amino-5-methylbenzoic acid (**4**, 2.52 g, 16.7 mmol) and carbon disulfide (2.01 mL, 33.9 mmol) in 45 mL of dry 1,4-dioxane was added NEt_3 (5.58 mL, 40.0 mmol) and the mixture were stirred under N_2 atmosphere at 5 °C for 18 h. Iodomethane (1.14 mL, 18.4 mmol) was added dropwise and the mixture was stirred at 5 °C for 1 h. The reaction was poured into 25 mL of an aqueous solution of HCl (1 M) and the mixture was concentrated to half its volume under reduced pressure and extracted three times with EtOAc (75 mL each time). The combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. The residue was recrystallized from chloroform to give **5** as a yellow solid (3.25 g, 81%). mp: 199 °C. ^1H NMR (400 MHz, MeOD-d4) δ 8.82 (1H, d, J = 8.2 Hz), 7.90 (1H, s), 7.38 (1H, d, J = 8.2 Hz), 2.63 (3H, s), 2.35 (3H, s); ^{13}C NMR (100 MHz, MeOD-d4) δ 189.2, 161.4, 130.9, 126.5, 125.4, 123.2, 114.2, 110.7, 11.3, 8.9; HRMS (ESI): for $C_{10}H_{12}NO_2S_2$ Calcd: 242.0309; [M+H]; found: 242.0316.

*2.1.2. 6-Methyl-2-(methylsulfanyl)-4H-3,1-benzothiazin-4-one (**6**)*

Compound **5** (2.85 g, 11.8 mmol) was dissolved in 50 mL of acetic anhydride and heated at reflux for 1 h. The mixture was cooled to room temperature and the precipitate was filtered off. The crude product was recrystallized from ethanol to give **6** as white needle-shaped crystals (2.17 g, 83%). mp: 114 °C. ^1H NMR (400 MHz, $CDCl_3$ + 5% MeOD-d4) δ 7.95 (1H, s), 7.57 (2H, bs), 2.70 (3H, s), 2.45 (3H, s); ^{13}C NMR (100 MHz, CDCl3 + 5% MeOD-d4) δ 183.5, 162.3, 146.3, 138.0, 137.1, 129.8, 124.7, 119.1, 21.3, 14.2; HRMS (ESI): for $C_{10}H_{10}NOS_2$ Calcd: 224.0204; [M+H]; found: 224.0201.

*2.1.3. Methyl {2-[(2Z)-3-hydroxypent-2-enoyl]-4-methylphenyl} dithiocarbamate (**7**)*

A solution of 1.6 M *n*-BuLi (0.78 mL, 1.25 mmol) in hexane was added to a solution of diisopropylamine (0.18 mL, 1.3 mmol) in 5 mL of THF under N_2 atmosphere at −78 °C. The solution was heated to 0 °C and stirred for 5 min and then once again cooled to −78 °C. To the resultant LDA solution was added a solution of butanone (0.112 mL, 1.25 mmol) in 2 mL of THF and the mixture was stirred for 1 h. A solution of **6** in 3 mL of THF was slowly added and the mixture was slowly heated to −40 °C over a period of 3 h, while monitored by TLC. The reaction was poured onto 3 mL of an aqueous solution of HCl (1 M) and the mixture was concentrated to half its volume. The residue was extracted once with 100 mL of EtOAc and the organic layer was washed with brine, dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by chromatography on silica gel column. Elution with *n*-heptane/toluene/acetone (50:50:1) as eluent yielded **7** (61%) as a yellow solid (mp 25 °C). ^1H NMR (400 MHz, $CDCl_3$) δ 11.70 (1H, s), 8.63 (1H, d, J = 8.4 Hz), 7.49 (1H, J = 1.7 Hz), 7.33 (1H, dd, J = 8.4 and 1.7 Hz), 6.09 (1H, s), 2.67 (3H, s), 2.43 (2H, q, J = 7.6 Hz), 2.38 (3H, s), 1.24 (3H, t, J = 7.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 198.4, 192.5, 191.7, 137.1, 135.2, 133.4, 129.5, 126.3, 124.4, 97.4, 30.6, 21.2, 18.7, 10.5; HRMS (ESI): for $C_{14}H_{18}NO_2S_2$ Calcd: 296.0779; [M+H]; found: 296.0763.

*2.1.4. Methyl {2-[(2Z)-3-hydroxyhept-2-enoyl]-4-methylphenyl} dithiocarbamate (**8**)*

Methyl {2-[(2*Z*)-3-hydroxyhept-2-enoyl]-4-methylphenyl} dithiocarbamate (**8**) was prepared and purified according to the

description for **7**, starting from 2-hexanone. The reaction yielded **8** (75%) as a yellow solid (mp 58 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ 15.64 (1H, s), 11.81 (1H, s), 8.66 (1H, d, J = 8.4 Hz), 7.48 (1H, J = 1.7 Hz), 7.31 (1H, dd, J = 8.4 and 1.7 Hz), 6.09 (1H, s), 2.67 (3H, s), 2.37 (5H, m), 1.66 (2H, m), 1.41 (2H, hex., J = 7.6 Hz), 0.96 (3H, t, J = 7.3 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 198.2, 192.6, 190.6, 137.2, 135.0, 133.4, 129.5, 126.0, 124.1, 98.1, 37.0, 28.5, 22.5, 21.1, 18.6, 13.9; HRMS (ESI): for $C_{16}H_{22}NO_2S_2$ Calcd: 324.1092; [M+H]; found: 324.1101.

2.1.5. Methyl {2-[(2Z)-3-hydroxyoct-2-enoyl]-4-methylphenyl} dithiocarbamate (**9**)

Methyl {2-[(2*Z*)-3-hydroxyoct-2-enoyl]-4-methylphenyl}dithio carbamate (**9**) was prepared and purified according to the description for **7**, starting from 2-heptanone. The reaction yielded **9** (51%) as a yellow solid (mp 63 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ 15.66 (1H, s), 11.79 (1H, s), 8.67 (1H, d, J = 8.4 Hz), 7.49 (1H, d, J = 1.6 Hz), 7.34 (1H, dd, J = 8.4 and 1.6 Hz), 6.10 (1H, s), 2.68 (3H, s), 2.39 (5H, m), 1.7 (2H, m), 1.37 (4H, m), 0.93 (3H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 198.3, 192.7, 190.7, 137.3, 135.1, 133.4, 129.6, 126.2, 124.3, 98.2, 37.4, 31.6, 26.2, 22.6, 21.2, 18.7, 14.1; HRMS (ESI): for $C_{17}H_{24}NO_2S_2$ Calcd: 338.1243; [M+H]; found: 338.1267.

2.1.6. 1-(4-Hydroxy-6-methyl-2-thioxo-1,2-dihydroquinolin-3-yl) propan-1-one (**10**)

To keto-enol **7** (91.3 g, 0.309 mmol) was added 5 mL of a 0.5 M solution of sodium methoxide in methanol and the mixture was stirred at 0 °C for 3 h. A 1.0 M solution of hydrochloric acid (3 mL) was poured onto the reaction and the mixture was concentrated to less than half its volume under reduced pressure and extracted three times with EtOAc. The combined organic layers were dried over $MgSO_4$, concentrated under reduced pressure and precipitated from MeOH to give **10** as a yellow solid (1.70 g, 97%). mp: 238 °C; ^{1}H NMR (400 MHz, DMSO-d6) δ 13.11 (1H, s), 12.27 (1H, s), 7.91 (1H, s), 7.52 (2H, s), 2.93 (2H, q, J = 7.1 Hz), 2.38 (3H, s), 1.09 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 204.2, 176.8, 156.7, 138.2, 133.9, 133.1, 123.7, 122.7, 117.0, 116.3, 36.4, 20.8, 7.9; HRMS (ESI): for $C_{13}H_{14}NO_2S$ Calcd: 248.0745; [M+H]; found: 248.0739.

2.1.7. 1-(4-Hydroxy-6-methyl-2-thioxo-1,2-dihydroquinolin-3-yl) pentan-1-one (**11**)

1-(4-Hydroxy-6-methyl-2-thioxo-1,2-dihydroquinolin-3-yl)pentan-1-one (**11**) was prepared and purified according to the description for **10**, starting from **8**. The reaction yielded **11** (97%) as a yellow solid (mp 170 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ 17.13 (1H, s), 11.18 (1H, s), 7.96 (1H, d, J = 1.7 Hz), 7.50 (1H, dd, J = 8.4 and 1.7 Hz), 7.27 (1H, d, J = 8.4 Hz), 3.76 (2H, t, J = 7.3 Hz), 2.45 (3H, s), 1.77 (2H, pent, J = 7.4 Hz), 1.47 (2H, hex, J = 7.4 Hz), 0.98 (3H, t, J = 7.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 211.5, 180.6, 173.0, 138.1, 136.9, 134.6, 125.0, 117.6, 115.9, 115.4, 44.5, 27.2, 22.6, 21.4, 14.3; HRMS (ESI): for $C_{15}H_{18}NO_2S$ Calcd: 276.1058; [M+H]; found: 276.1072.

2.1.8. 1-(4-Hydroxy-6-methyl-2-thioxo-1,2-dihydroquinolin-3-yl) hexan-1-one (**12**)

1-(4-Hydroxy-6-methyl-2-thioxo-1,2-dihydroquinolin-3-yl) hexan-1-one (**12**) was prepared and purified according to the description for **10**, starting from **9**. The reaction yielded **12** (96%) as a yellow solid (mp 150 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ 17.19 (1H, s), 11.39 (1H, s), 7.99 (1H, bs), 7.54 (1H, dd, J = 8.4 and 1.6 Hz), 7.32 (1H, d, J = 8.4 Hz), 3.67 (2H, t, J = 7.3 Hz), 2.45 (3H, s), 1.79 (2H, pent, J = 7.3 Hz), 1.41 (4H, m), 0.94 (3H, t, J = 7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 211.3, 179.7, 173.1, 138.1, 137.0, 134.9, 124.9, 117.7, 115.7, 115.6, 44.7, 31.7, 24.8, 22.8, 21.4, 14.2; HRMS (ESI): for $C_{16}H_{20}NO_2S$ Calcd: 290.1209; [M+H]; found: 290.1209.

2.1.9. 3-Ethyl-6-methylisothiazolo[5,4-b]quinolin-4(9H)-one (**13**)

To a solution of **10** (64 mg, 0.259 mmol) in 25 mL of methanol was added a solution of hydroxylamine-*O*-sulfonic acid (102.6 mg, 0.907 mol) and lithium hydroxide (38.1 mg, 0.907 mmol) in 3 mL of methanol and the mixture was stirred at room temperature for 30 h. The reaction mixture was concentrated under reduced pressure and applied to flash chromatography. Elution with heptane/EtOAc (3:1) yielded **13** (63%) as white crystals (mp 332 °C). ^{1}H NMR (400 MHz, DMSO-d6) δ 12.70 (1H, s), 8.03 (1H, d, J = 2.0 Hz), 7.58 (1H, dd, J = 8.4 and 2.0 Hz), 7.43 (1H, d, J = 8.4 Hz), 3.17 (2H, q, J = 7.4 Hz), 2.43 (3H, s), 1.26 (3H, t, J = 7.4 Hz); ^{13}C NMR (100 MHz, DMSO-d6) δ 173.4, 170.3, 165.8, 138.1, 134.4, 132.3, 125.3, 123.3, 118.0, 117.5, 27.1, 20.6, 12.0; HRMS (ESI): for $C_{13}H_{13}N_2OS$ Calcd: 245.0749; [M+H]; found: 245.0747.

2.1.10. 3-Butyl-6-methylisothiazolo[5,4-b]quinolin-4(9H)-one (**14**)

3-Butyl-6-methylisothiazolo[5,4-*b*]quinolin-4(9*H*)-one (**14**) was prepared and purified according to the description for **13**, starting from **11**. The reaction yielded **14** (82%) as white crystals (mp 243 °C). ^{1}H NMR (400 MHz, DMSO-d6) δ 12.72 (1H, s), 8.02 (1H, s), 7.56 (1H, d, J = 8.4 Hz), 7.41 (1H, d, J = 8.4 Hz), 3.15 (2H, t, J = 7.7 Hz), 2.42 (3H, s), 1.69 (2H, pent, J = 7.5 Hz), 1.36 (2H, hex, J = 7.4 Hz), 0.91 (3H, t, J = 7.4 Hz); ^{13}C NMR (100 MHz, DMSO-d6) δ 173.4, 169.3, 165.7, 138.0, 134.4, 132.2, 125.3, 123.5, 118.1, 117.4, 33.1, 29.6, 21.9, 20.6, 13.8; HRMS (ESI): for $C_{15}H_{17}N_2OS$ Calcd: 273.1062; [M+H]; found: 273.1077.

2.1.11. 6-Methyl-3-pentylisothiazolo[5,4-b]quinolin-4(9H)-one (**15**)

6-Methyl-3-pentylisothiazolo[5,4-*b*]quinolin-4(9*H*)-one (**15**) was prepared and purified according to the description for **13**, starting from **12**. The reaction yielded **15** (93%) as white crystals (mp 245 °C). ^{1}H NMR (400 MHz, DMSO-d6) δ 12.71 (1H, s), 8.03 (1H, s), 7.57 (1H, dd, J = 8.4 and 2.0 Hz), 7.42 (1H, d, J = 8.4 Hz), 3.14 (2H, m), 2.42 (3H, s), 1.71 (2H, m), 1.33 (4H, m), 0.87 (3H, m); ^{13}C NMR (100 MHz, DMSO-d6) δ 173.4, 169.4, 165.8, 138.0, 134.4, 132.2, 125.3, 123.5, 118.2, 117.4, 33.4, 31.1, 27.2, 21.9, 20.6, 13.9; HRMS (ESI): for $C_{16}H_{19}N_2OS$ Calcd: 287.1213; [M+H]; found: 287.1210.

2.1.12. Methyl [2-(cyanoacetyl)-4-methylphenyl]dithiocarbamate (**16**)

A solution of 1.6 M *n*-BuLi (5.4 mL, 13.4 mmol) in hexane was added to a solution of diisopropylamine (1.95 mL, 14.0 mmol) in 20 mL of THF under N_2 atmosphere at −78 °C. The solution was heated to 0 °C and stirred for 5 min and then once again cooled to −78 °C. To the resultant LDA solution was added acetonitrile (0.28 mL, 13.4 mmol) and the mixture was stirred for 1 h at −20 °C. A solution of **6** (1.20 g, 5.4 mmol) in 8 mL of THF was slowly added at −78 °C and the mixture was stirred for 1 h. The reaction was poured onto 40 mL of an aqueous solution of HCl (1 M) and the mixture was concentrated to half its volume. The residue was extracted with EtOAc and the organic layer was washed with brine, dried over $MgSO_4$, concentrated and crystallized from ethyl alcohol to give **16** as a yellow solid (1.41 g, 99%). mp: 142 °C; ^{1}H NMR (400 MHz, DMSO-d6) δ ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.89 (1H, s), 9.00 (1H, d, J = 8.3 Hz), 7.50 (1H, s), 7.48 (1H, d, J = 8.3 Hz), 4.17 (2H, s), 2.41 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 199.3, 191.0, 139.2, 136.7, 135.0, 130.6, 123.5, 122.1, 113.5, 31.2, 21.2, 18.7; HRMS (ESI): for $C_{12}H_{13}N_2OS_2$ Calcd: 265.0469; [M+H]; found: 265.0483.

*2.1.13. 4-Hydroxy-6-methyl-2-thioxo-1,2-dihydroquinoline-3-carbonitrile (**17**)*

4-Hydroxy-6-methyl-2-thioxo-1,2-dihydroquinoline-3-carbonitrile (**17**) was prepared and purified according to the description for **10**, starting from **16**. The reaction yielded **17** (97%) as a yellow solid (mp 284 °C). ^{1}H NMR (400 MHz, DMSO-d6) δ 12.89 (1H, s), 7.85 (1H, s), 7.51 (1H, d, J = 8.3 Hz), 7.44 (1H, d, J = 8.3 Hz), 2.36 (3H, s); ^{13}C NMR (100 MHz, DMSO-d6) δ 171.2, 164.9, 138.2, 135.1, 134.7, 123.7, 121.0, 118.1, 116.0, 98.8, 20.8; HRMS (ESI): for $C_{11}H_9N_2OS$ Calcd: 217.0436; [M+H]; found: 217.0439.

*2.1.14. N-(6-Methyl-4-oxo-4,9-dihydroisothiazolo[5,4-b]quinolin-3-yl)propanamide (**18**)*

To a solution of **17** (30 mg, 0.139 mmol) in 1 mL of DMF was added a solution of $NaHCO_3$ (41 mg, 0.43 mmol) and hydroxylamine-*O*-sulfonic acid (55 mg, 0.43 mmol) in 1 mL of methyl alcohol and the mixture was stirred for 2 h, during which a white precipitate presumed to be the aminothioisoxazole was formed. This was filtered off and slurried in 2 mL of DMF. Propionyl chloride (19.2 μL, 0.221 mmol) and 0.10 mL of pyridine was added and a clear solution was formed. The mixture was stirred for 5 h, concentrated and purified by chromatography. Elution with *n*-heptane/EtOAc (1:1) afforded **18** (19%) as a white solid [mp 300 °C (decomp)]. ^{1}H NMR (400 MHz, DMSO-d6) δ 11.62 (1H, s), 9.80 (1H, s), 7.86 (1H, d, J = 2 Hz), 7.74 (1H, dd, J = 8.4 and 2 Hz), 7.59 (1H, d, J = 8.4 Hz), 2.41 (5H, m), 1.07 (3H, t, J = 7.4 Hz); ^{13}C NMR (100 MHz, DMSO-d6) δ 174.2, 171.2, 164.0, 153.0, 138.2, 135.2, 133.3, 124.7, 122.4, 118.0, 110.0, 28.2, 20.8, 9.8; HRMS (FAB+): for $C_{14}H_{14}N_3O_2S$ Calcd: 288.0807; [M+H]; found: 288.0832.

*2.1.15. N-(6-Methyl-4-oxo-4,9-dihydroisothiazolo[5,4-b]quinolin-3-yl)butanamide (**19**)*

N-(6-Methyl-4-oxo-4,9-dihydroisothiazolo[5,4-*b*]quinolin-3-yl) butanamide (**19**) was prepared and purified according to the description for **18**, starting from **17**, with butyryl chloride (23.1 μL, 0.221 mmol) instead of propionyl chloride. The reaction yielded **19** as a white solid [19 mg, 45%, mp 320 °C (decomp)]. ^{1}H NMR (400 MHz, DMSO-d6) δ 12.93 (1H, s), 10.74 (1H, s), 8.00 (1H, d, J = 2.0 Hz), 7.62 (1H, dd, J = 8.4 and 2.0 Hz), 7.47 (1H, d, J = 8.4 Hz), 2.60 (2H, J = 7.4 Hz), 2.42 (3H, s), 1.65 (2H, hex, J = 7.4 Hz), 0.95 (3H, t, J = 7.4 Hz); ^{13}C NMR (100 MHz, DMSO-d6) δ 174.2, 170.9, 164.1, 153.3, 138.5, 135.2, 133.2, 124.7, 122.6, 118.1, 109.8, 38.7, 20.7, 18.0, 13.7; HRMS (FAB+): for $C_{15}H_{16}N_3O_2S$ Calcd: 302.0963; [M+H]; found: 302.0959.

2.2. Benzodiazepine receptor binding in vitro

The binding of ^{3}H-flumazenil (87 Ci/mmol) to rat cortical membranes was done following the methods previously described in detail by Dekermendjian et al. [3]. 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 27,000*g* 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 the concentration 50 μg protein/0.55 mL assay (1 mg original tissue/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 to 5 mL ice-cold buffer (Tris, citrate, 50 mM pH 7.1), the suspension was poured directly onto Whatman GF/C glass fiber 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 by assaying 4–6 different concentrations of each 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).

3. Results

3.1. Chemical synthesis

All isothiazolo[5,4-*b*]quinolin-4(9*H*)-ones prepared and presented in this investigation are to our knowledge new compounds. For all target compounds, an unambiguous structure determination was performed using COSY, HMQC, HMBC and NOESY NMR experiments as well as high resolution mass spectrometry.

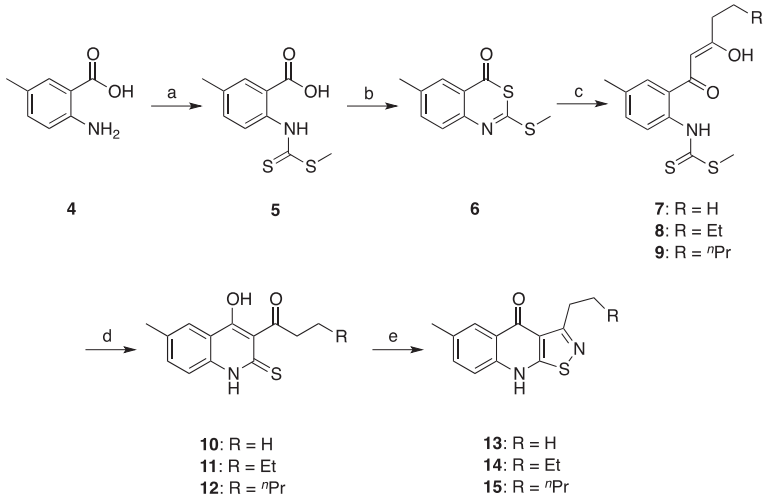
The key intermediate for the preparation of the isothiazolo[5,4-*b*]quinolin-4(9*H*)-one derivatives **13**, **14**, **15**, **18** and **19** is the 4*H*-benzo[*d*][1,3]thiazin-4-one **6**, which was synthesized by the addition of carbon disulfide to the anthranilic acid **4** followed by methylation of the sulfur and thiolactone formation in acetic anhydride (Scheme 1) [9]. The addition of different 2-alkanones to **6** afforded the keto-enols **7**–**9**, which were recyclized to give the corresponding thione derivatives **10**–**12**. Treatment of the thiones **10**–**12** with hydroxylamine-*O*-sulfonic acid afforded the 3-alkyl-6-methyl-isothiazolo[5,4-*b*]quinolin-4(9*H*)-ones **13**–**15** [10] (Scheme 1). The 3-amido-6-methylisothiazolo[5,4-*b*]quinolin-4(9*H*)-ones **18** and **19** were prepared from the cyano derivative **16**, prepared by the addition of acetonitile to **6** (Scheme 2) following essentially the same protocol as for the synthesis of **7**, and cyclized to **17**. The amination of the thione functionality of **17** with hydroxylamine-*O*-sulfonic acid yielded the aminothioisoxazole, which due to limited chemical stability was not isolated but acylated directly with propionyl chloride and butyryl chloride to yield **18** and **19**, respectively. Cromatographic and spectroscopic investigations of the degradation products of the aminothioisoxazole formed from **17** suggested that it gradually polymerized in DMF.

3.2. Receptor binding

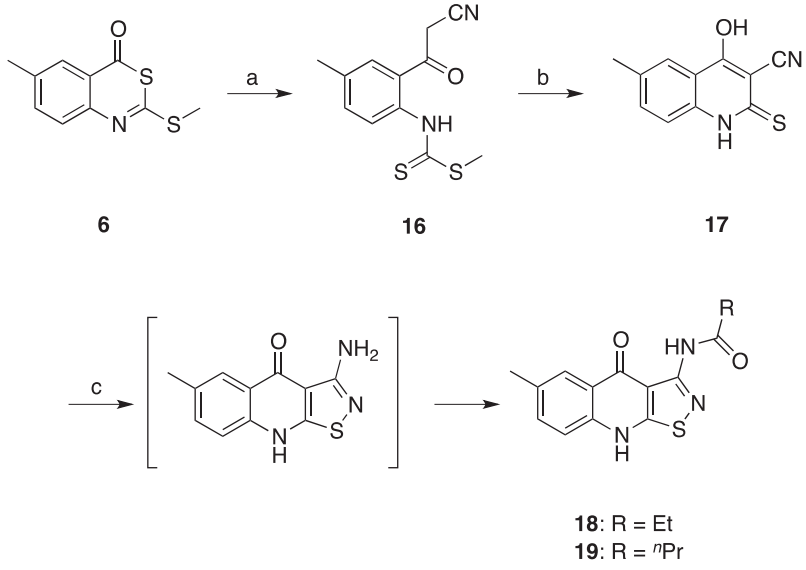
Affinities for the BZD binding site were determined with the same *in vitro* radio ligand assay that was used in our previous investigations, by the displacement of [^{3}H]-flumazenil in rat cortical tissue (see Experimental for details). The results are presented in Table 1.

4. Discussion

The pharmacophore model for ligands of the benzodiazepine binding site of the $GABA_A$ receptors has been used and validated for the design of several active compound classes [5,6], and again it is demonstrated that a structure that fits into the model and fulfills the requirements discussed below will display affinity for the benzodiazepine binding site. This is shown in Fig. 3, where 3-pentyl-6-methylisothiazoloquinolin-4-one (**15**) (left) and 3-butyramido-6-methyl isothiazoloquinolin-4-one (**19**) (right) are positioned into the pharmacophore model. The interactions between all hydrogen bond donator/acceptor sites are satisfied, and no steric repulsive ligand-receptor interaction is indicated. The small difference in the K_i value for **15** and **19** noted, approximately five times, may not completely reflect the difference in affinity of the two compounds to the binding site as **19** should be considerably more



Scheme 1. Conditions: (a) CS_2, NEt_3, dioxane, 5 °C, 18 h, then MeI, 5 °C, 1 h, yield 81%; (b) Ac_2O, reflux, 1 h, yield 83%; (c) LDA, 2-butanone for **7**,2-hexanone for **8**, or 2-heptanone for **9**, THF, −78 °C, 1 h, then **6**, −78 °C to −40 °C, 3 h (yields 61% for **7**, 75% for **8**, and 51% for **9**); (d) NaOMe, MeOH, 0 °C, 3 h, yield 97% for **10**, **11** and **12**); (e) H_2NOSO_3H, LiOH, MeOH, rt, 24 h (yield 63% for **13**, 82% for **14**, and 93% for **15**).



Scheme 2. Conditions: (a) LDA, MeCN, THF, −78 °C, 1 h, then **6**, −20 °C, 1 h, yield 99%; (b) NaOMe, MeOH, 0 °C, 3 h, yield 97%; (c) H_2NOSO_3H, $NaHCO_3$, MeOH, DMF, rt., 2 h, then CH_3CH_2COCl for **18** or $CH_3(CH_2)_2COCl$ for **19**, pyridine, DMF, 5 h (yield 19% for **18** and 45% for **19**.

Table 1
K_i values of isothiazoloquinolin-4-ones **13**, **14**, **15**, **18** and **19** tested on ^{3}H-flumazenil binding *in vitro* to rat cortical membranes.

Compd	K_i (nM)[a]
±**13**	930 ± 120
14	56 ± 2
15	13 ± 1.7
18	121 ± 9
19	2.8 ± 0.7

[a] Each K_i value is the mean of ±SD of three determinations.

hydrophilic compared to **15**. Nevertheless, it is reasonable to assume that **19** is able to bind stronger that **15** due to the extra interaction between the amido oxygen and H1 as indicated in Fig. 3 (right), possibly directed by an internal hydrogen bond between the amido NH and the 4-carbonyl oxygen. However, it cannot be excluded that this additional interaction actually take place between the amido oxygen and H2, and it should be noted that the 3-butyl derivative **14** has a slightly higher affinity than the propionamido derivative **18**.

In addition, as has been demonstrated during the development of both the flavones and the quinolin-4-ones, an efficient filling of the L2 lipophilic pocket may influence the affinity considerably. This is confirmed in this investigation, both for the 3-alkyl and the 3-amido derivatives. Previous investigations (flavones as well as quinolin-4-ones) have very clearly shown that the space available at L2 is quite limited and too big substituents in position 3 will diminish the affinity. For the quinolin-4-ones, nothing bigger than the 3-substituents in compounds **1**, **2** and **3**, i.e. a 3-valeryl, a 3-propyloxycarbonyl or a 3-(*N*-propylcarbamoyl), will be accepted by the binding site, independent if it is a longer chain length, a branched chain, or a cycloalkyl group. We are therefore fairly certain that the pentyl derivative **15** and the butyroamide derivative **19** are the most potent of these two compound classes.

Another requirement for ligands of the benzodiazepine site fulfilled by the isothiazoloquinolin-4-ones concerns their three dimensional form, as previous studies have emphasized that li-

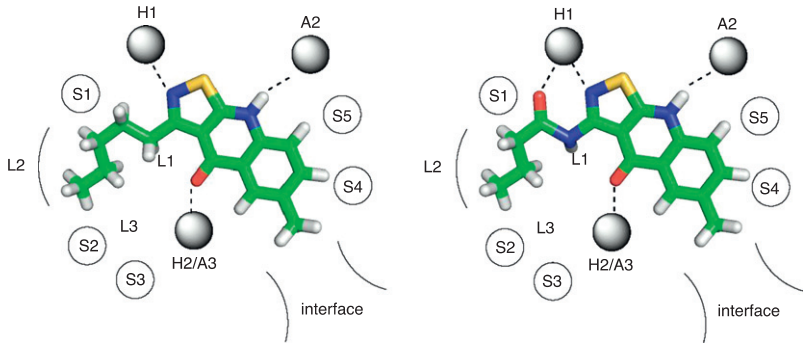


Fig. 3. Left: 3-Pentyl-6-methylisothiazoloquinolinone (**15**) positioned in the model. Right: 3-Butyramido-6-methylisothiazoloquinolinone (**19**) positioned in the model.

gands with a strong affinity for this binding site must be able to adopt a planar or close to planar conformation [2]. There are obviously no intramolecular interactions possible in the compounds assayed in this investigation that will prevent them from being essentially planar.

5. Conclusion

The usefulness of the pharmacophore model describing the binding site for benzodiazepines in the GABAA receptors was again demonstrated, by the design, synthesis and testing of 3-alkylisothiazolo[5,4-*b*]quinolin-4(9*H*)-ones and 3-amidoisothiazolo[5,4-*b*]quinolin-4(9*H*)-ones. The most potent compounds prepared have high affinity with K_i values in the low nM range. The difference between the most potent 3-alkylisothiazoloquinolin-4-one (**15**) and the most potent 3-amidoisothiazoloquinolin-4-one (**19**) is suggested to depend on the additional interaction with the H1 site of the pharmacophore model made possible by the amido oxygen.

Acknowledgments

Financial support from the Swedish board for Scientific research (VR), the Knut and Alice Wallenberg Foundation, the research School for Pharmaceutical Sciences at Lund University, Carlsberg Foundation, Denmark, and the NeuroScience PharmaBiotec Research Center, Denmark, is gratefully acknowledged.

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