Synthesis of Thieno[2,3-*b*]Pyridinones Acting as Cytoprotectants and as Inhibitors of [^{3}H]Glycine Binding to the *N*-Methyl-D-aspartate (NMDA) Receptor

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Received April 14, 2005

The standard glycine site antagonist of the *N*-methyl-D-aspartate (NMDA) receptor, 3-phenyl-4-hydroxyquinolin-2(1*H*)-one (**21**), was used as a template for bioisostere benzene/thiophene exchange. Phenylacetylation of aminothiophene carboxylic acid methyl esters and subsequent cyclization delivered the three possible thienopyridinone isomers. 4-Hydroxy-5-phenylthieno[2,3-*b*]pyridin-6(7*H*)-one (**3a**), with the shortest distance between the sulfur and the nitrogen atom, was the most potent isomer (K_i against the binding of [^{3}H]glycine to rat membranes 16 μM), comparable in potency to the model quinolinone (**21**, 12 μM). Replacement of the phenyl substituent of **21** by a 2-thienyl residue resulted in a 2−5-fold loss in potency and was abandoned. In the thieno part of the thienopyridinone nucleus, the most successful substituents were halogen (Cl or Br) close to the sulfur atom and short alkyl chains at the other position, resulting in **7h**, **8h**, **8i**, and **8m**, with K_i values between 5.8 and 10.5 nM. Introduction of a 3′-phenoxy moiety yielded several compounds with still higher potencies (**18h**, **18i**, **18l**, and **18m**; K_i between 1.1 and 2.0 nM). Quantitative structure−activity relationship (QSAR) calculations resulted in a consistent interpretation of the potencies of most compounds. Several of these 3′-phenoxy derivatives protected mouse fibroblast cell lines with transfected NMDA receptors from glutamate-induced toxicity. In addition, we report in vivo results for four of these compounds.

Introduction

N-Methyl-D-aspartate (NMDA) receptors (NRs) are ligand-gated ion channels with a high permeability for Ca^{2+}. They contribute to excitatory neurotransmission[1] and synaptic plasticity[2] as well as to neurodegenerative diseases[3] like stroke, epilepsy, Parkinson's disease, Huntington's chorea, Alzheimer's disease, and HIV dementia. The endogenous amino acid glycine acts as a co-agonist at the NR,[4] and the glycine binding site of the NR has become a target for drug development.[5,6]

Immediately following the discovery of the glycine site of the NR,[7] it was recognized that kynurenic acid acts as an antagonist at this site.[8] In addition, many derivatives of 2,3-dihydroxyquinoxaline (QX), originally introduced as antagonists of non-NMDA receptors, were subsequently shown to block the glycine site of the NR.[9] For the present study, a series of 2-quinolone derivatives was designed with the goal of combining structural features present in kynurenic acids and QX. Quinolones with a phenyl substituent vicinal to the keto function[10] belong to the most potent glycine antagonists yet described, both in vitro and in vivo (e.g., L-701,324; see ref 5 for review). These compounds lack the carboxylic acid function of kynurenic acid; it is supposed that the anionic functionality, which is essential for binding, is contributed by an electron excess in the phenyl substituent.

The aim of this work is to present synthetic protocols and results of binding assays for a series of thieno[2,3-*b*]pyridin-6(7*H*)-one derivatives, derived from quinolones by replacement of the benzene nucleus by thiophene (for structures see Chart 1). Binding affinities were determined in a competition binding assay with the radioligand [^{3}H]glycine and rat brain membranes. To demonstrate the in vitro cell protective potency of the compounds, we used the fibroblast cell line L(tk$^-$) stably transfected with the recombinant human NR subunits NR1a and NR2A in a cell toxicity assay.[11] Finally, we present in vivo data on four of our most potent compounds.

Bioisosterism. The pair benzene/thiophene represents one of the most prominent examples of bioisosterism: the exploratory replacement in successful drugs of a benzene by a thiophene moiety has become a routine strategy in modern drug design. Both aromatic rings are similar in size (isostere) and electronic properties. The diameter of the sulfur atom in thiophene is roughly equivalent to the distance between two neighboring carbon atoms in benzene. The physiological effects of thiophene are similar to those of benzene (bioisostere), with frequently superior pharmacodynamic, pharmacokinetic, or toxicological properties. Finally, the three different fusion patterns in thiophene allow for a fine-tuning not accessible in benzene derivatives (Chart 1). When the three positions of the sulfur atom in the three thienopyridinone isomers **3a**, **3b**, and **3c** are taken into account, each isomer allows attachment of two residues with slightly differing orientations. By this synthetic strategy, six potential pharmacophore positions can be addressed, whereas the single benzo[*b*]pyridinone isomer addresses only four such positions. Thus, thiophene-containing ligands may become sublime tools to investigate receptor binding pockets.[12]

Chemistry. For the preparation of all compounds described in this paper, the appropriate substituted aminothiophenes were required as starting materials. Compounds **1a**−**c**, which were necessary for the syntheses of the different isomeric thienopyridinones **3a**−**c**, were either obtained according to the method of Gewald et al.[13] (**1a**) or commercially available (**1b**, **1c**). The syntheses of compounds **3a**−**c** were accomplished in two steps as depicted in Scheme 1. Acylation of **1a**−**c** with a small excess

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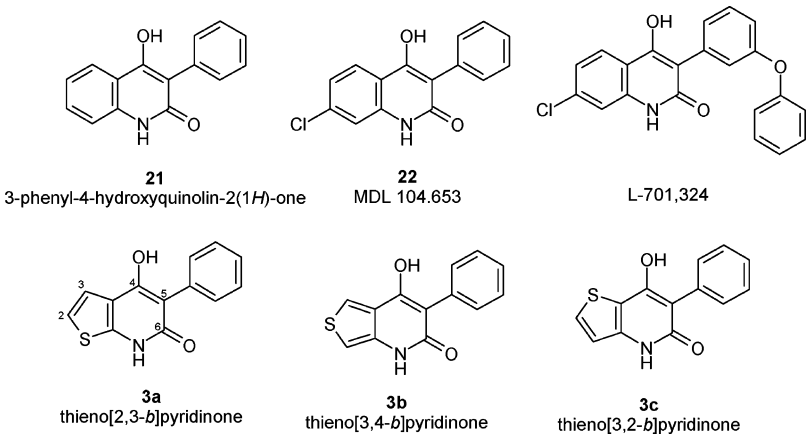
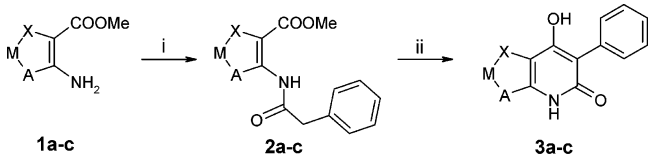
10.1021/jm0503493 CCC: $33.50
Published on Web 01/06/2006

Chart 1



Scheme 1[a]



1a-3a: A = S; M,X = CH
1b-3b: A, X = CH; M = S
1c-3c: A, M = CH; X = S

[a] Reagents: (i) $PhCH_2COCl$, dioxane; (ii) $KN(Si(CH_3)_3)_2$, THF.

of phenylacetyl chloride in dry dioxane at high temperature provided the intermediate amides **2a**−**c** in excellent yields.[14] Subsequent cyclization in THF at low temperature with potassium hexamethyldisilazide afforded the desired thienopyridine derivatives. Substituted 2-aminothiophenes (**1d**−**g**, **1s**−**u**) with the appropriate substitution pattern at C-2 and C-3, which gave access to further thieno[2,3-*b*]pyridin-6(7*H*)-one derivatives, were synthesized according to the methods of Gewald et al.[13] or by Gewald-related preparations (**1h**−**r**, **1v**, **1w**), which have been described elsewhere.[15] Following the acylation conditions described above, aminothiophenes **1d**−**i**, **1k**, **1m**, and **1q** were converted to the corresponding intermediates **2a**−**i**, **2k**, **2m**, **2q**, **2t**, **11d**, **11g**, and **11t**. The amides **2a**−**i**, **2k**, **2m**, **2t**, **11d**, **11g**, and **11t** were cyclized to thienopyridines **3a**−**i**, **3k**, **3m**, **3t**, **12d**, **12g**, and **12t**, as outlined in Scheme 2. Compounds **2g**−**i**, **2k**, **2m, and 2q**, which bear no substituent at the 5-position (R_1 in Scheme 2), were halogenated in refluxing chloroform with *N*-bromosuccinimide (NBS) or *N*-chlorosuccinimide (NCS), respectively. The subsequent cyclization of halogenated derivatives **4g**, **4h**, **5g**−**i**, **5k**, **5m**, and **5q** was accomplished with potassium hexamethyldisilazide at low temperature. Compounds **7g**, **7h**, **8g**−**i**, **8k**, and **8m** were obtained in moderate yields, while the cyclization of **5q** to **8q** failed. Alternatively, **8q** could be obtained at least in a low yield when phosphazene-P_2-*t*-Bu was used for deprotonation. The synthesis of halogen-substituted derivatives of **3a** via the two-step procedure failed, because decomposition was observed during cyclization. However, **7a** could be obtained from **3a** by subsequent bromination with bromine in glacial acetic acid. Thieno[2,3-*b*]pyridin-6-thione **10** was obtained from **5i**, which was selectively converted into the thioamide **6** with 1 equiv of Lawesson's reagent [2,4-bis(4-methoxyphenyl)-1,3-dithia-2,4-diphosphetane-2,4-disulfide] in refluxing dioxane, followed by cyclization to **10** under the typical conditions.

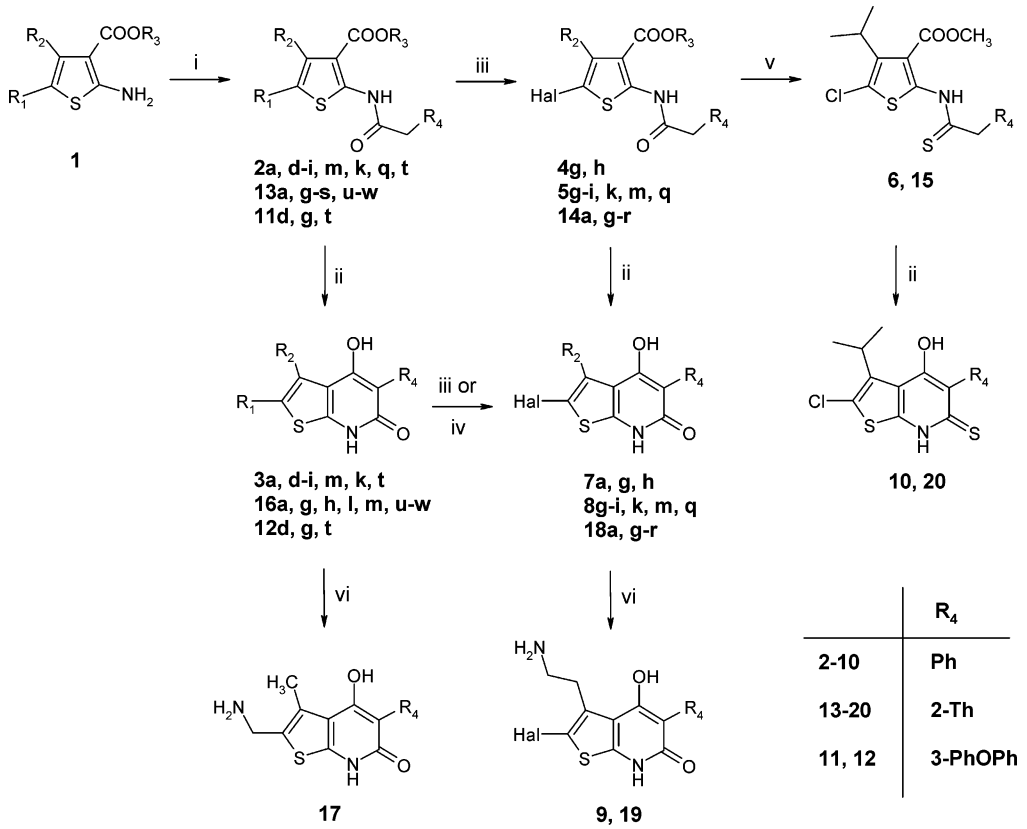
The syntheses of thienopyridine derivatives **16a**, **16g**, **16h**, **16l**, **16m**, **16s**, **16u**−**w**, **18a**, **18g**−**r**, and **20**, which bear a phenoxyphenyl substituent at position 5, were performed by an analogous two- or three-step strategy, respectively, as outlined in Scheme 2. The corresponding amides of aminothiophenes **1a**, **1q**−**s**, **1u**, and **1w** were obtained by Mukaiyama coupling with 3-phenoxyphenylacetic acid in dichloromethane (DCM). Alternatively, aminothiophenes **1g**−**p** and **1v** were treated with 3-phenoxyphenylacetyl chloride in dry dioxane at elevated temperature. Both methods gave access to the intermediate amides **13a**, **13g**−**s**, and **13u**−**w** in excellent yields. Compounds **13a** and **13g**−**r**, which bear no substituent at the 5-position, were chlorinated in refluxing chloroform with NCS to produce compounds **14a** and **14g**−**r**. Finally, cyclization of compounds **13a**, **13g**, **13h**, **13l**, **13m**, **13s**, **13u**−**w**, and the chlorinated derivatives **14a** and **14g**−**r** with potassium hexamethyldisilazide resulted in thienopyridines **16a**, **16g**, **16h**, **16l**, **16m**, **16s**, **16u**−**w**, and **18a**, **18g**−**p**, and **18r** in moderate to good yields, while the cyclization of **14q** to **18q** failed. But alternatively, **18q** could be obtained in a low yield when phosphazene-P_2-*t*-Bu was used for deprotonation. In some cases (**13s**, **13w**, **14v**) lithium diisopropylamide (LDA) was used instead of potassium hexamethyldisilazide to afford the final compounds **16s**, **16w**, and **18v** at least in low yield. Compound **18l** could also be obtained from **16l** by subsequent chlorination with NCS in refluxing chloroform. This alternative route provided **18l** in 58% yield and improved the overall yield by 3-fold. Thieno[2,3-*b*]-pyridin-6-thione **20** was obtained from **14i**, which was selectively converted into the thioamide **15** with 1 equiv of Lawesson's reagent in refluxing dioxane, followed by cyclization to **20** under the typical conditions.

L-701,324 (Chart 1), which was used as reference compound to validate the binding assay, was synthesized in three steps with an overall yield of 10.5%. In short, 2-amino-4-chlorobenzoic acid was esterified in refluxing methanol containing hydrogen chloride. Subsequent acylation with 3-phenoxyphenylacetic acid followed the Mukaiyama protocol, followed by cyclization with potassium hexamethyldisilazide at low temperature in THF. 4-Hydroxy-3-phenylquinolin-2(1*H*)-one[10] (**21**) and 7-Cl-4-hydroxy-3-phenylquinolin-2(1*H*)-one[16] (**22**) were prepared according to the literature.

Results and Discussion

The comparison of the three isomeric thienopyridinones **3a**, **3b**, and **3c** showed that thieno[2,3-*b*]pyridinone **3a** and thieno-[3,4-*b*] **3b** yielded the best K_i values for blocking the binding of [^{3}H]glycine at rat brain membranes [Table 1, not significantly different in potency from the unsubstituted benzo[*b*]pyridinone **21**, by analysis of variance (ANOVA)]. Thereafter, numerous derivatives of the isomer **3a** were synthesized. Right from the

Scheme 2[a]



[a] Reagents: (i) $PhCH_2COCl$, dioxane; or 2-$ThCH_2COCl$, dioxane; or $PhCH_2COOH$, (chloromethyl)pyridinium iodide, DCM; or 3-$PhOPhCH_2COCl$, dioxane; or 3-$PhOPhCH_2COOH$, $SOCl_2$, dioxane; or 3-$PhOPhCH_2COOH$, (chloromethyl)pyridinium iodide, DCM; (ii) $KN(Si(CH_3)_3)_2$, THF; or LDA, THF; or phosphazene-P_2-*t*-Bu, THF; (iii) NBS or NCS, chloroform; (iv) Br_2, AcOH; (v) Lawesson's reagent, dioxane; (vi) HCl, EtOAc.

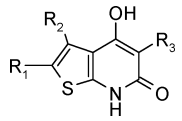
Table 1. Inhibition of [^{3}H]Glycine Binding by the Compounds Illustrated in Chart 1

	[^{3}H]glycine binding K_i (μM) ± SD (n)[a]
3a	16.4 ± 2.7 (6)
3b	26.0 ± 13.0 (3)
3c	97.5 ± 31.5 (3)
21	12.2 ± 5.2 (4)
22	0.151 ± 0.045 (5)
L-701,324	0.0014 ± 0.0002 (4)

[a] Number of experiments.

beginning of this first series, we wanted to investigate the option of another benzene/thiophene exchange, namely, at position 5. Three different 5-thienyl analogues **12d**, **12g**, and **12t** were synthesized in parallel to the respective phenyl analogues **3d**, **3g**, and **3t**, and all of them were considerably weaker than their 5-phenyl bioisosteres (see Table 2). As can be seen from the Free−Wilson analysis, the activity of the 5-thienyl analogues drops by a factor of 5.4 (0.73 log unit). Consequently, no further 5-thienyl derivatives were synthesized. From the quinolinone parent compound, it is known that lipophilic substituents in the benzene part of the bicyclic aromatic system enhance binding affinity. Similarly, alkyl substitution at both positions of the thiophene ring of **3a** resulted in compounds with enhanced potency. A single methyl group increased potency about 2-fold ($p < 0.01$, ANOVA), independent of whether it was attached to position 2 or to position 3 (see Table 2, **3d** and **3g**). A single ethyl group, however, increased the potency of **3a** by a factor of 4 in position 2 (**3e**) but by a factor 25 in position 3, returning with **3h** the first inhibitor with submicromolar potency in our series. Stepwise increase of the size of the R_3 substituent beyond that of an ethyl group did not improve potency any further (see Table 2, **3h**−**3m**). Flexible isopropyl (**3i**) and secondary butyl

Table 2. Inhibition of [^{3}H]Glycine Binding by 5-Phenyl- and 5-(2-Thienyl)thienopyridinones: Influence of Alkyl Substitution

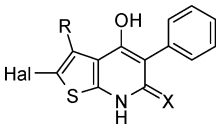


	R_1	R_2	R_3	[^{3}H]glycine binding K_i (μM) ± SD (n)[a]
3d	CH_3	H	Ph	9.5 ± 3.2 (3)
12d	CH_3	H	2-Th	22.7 ± 2.4 (3)
3e	C_2H_5	H	Ph	3.1 ± 3.0 (6)
3f	CH_3	CH_3	Ph	1.27 ± 0.48 (3)
3g	H	CH_3	Ph	8.3 ± 1.4 (3)
12g	H	CH_3	2-Th	46.0, 34.0 (2)
3h	H	C_2H_5	Ph	0.53 ± 0.29 (8)
3i	H	*i*-C_3H_7	Ph	1.53 ± 0.30 (3)
3k	H	*c*-C_3H_5	Ph	4.75 ± 0.55 (3)
3m	H	*sec*-C_4H_9	Ph	0.94 ± 0.05 (3)
3t		-$(CH_2)_4$-	Ph	4.3 ± 2.7 (3)
12t		-$(CH_2)_4$-	2-Th	23.1 ± 8.3 (3)

[a] Number of experiments.

(**3m**) substituents were almost as favorable as ethyl (**3h**), but the more rigid cyclopropyl residue (**3k**) missed the ethyl result by a factor of 9 ($p < 0.001$). Almost as powerful as these latter compounds was **3f**, with methyl groups at both positions 2 and 3. Thus, although a substituent as large as an ethyl group was not as welcome in position 2 as in position 3, a smaller size occupant also close to the sulfur atom appeared very promising. Joining both positions with a chain of four methylene groups, annulating a six-membered ring to the thiophene ring (**3t**), was less successful than two single methyl groups (**3f**, $p < 0.01$). Apparently, position 2 is confronted with some size limitation,

Table 3. Inhibition of [^{3}H]Glycine Binding by 5-Phenylthienopyridinones: Influence of Halogen and Alkyl Substitution



	Hal	R	X	[^{3}H]glycine binding K_i (μM) ± SD (n)[a]
7a	Br	H	O	1.77 ± 0.28 (3)
7g	Br	CH_3	O	0.39 ± 0.17 (5)
7h	Br	C_2H_5	O	0.0105 ± 0.0059 (3)
8g	Cl	CH_3	O	0.267 ± 0.116 (3)
8h	Cl	C_2H_5	O	0.0089 ± 0.0024 (5)
8i	Cl	*i*-C_3H_7	O	0.0066 ± 0.0038 (4)
10	Cl	*i*-C_3H_7	S	0.89 ± 0.16 (4)
8k	Cl	*c*-C_3H_5	O	0.058 ± 0.017 (3)
8m	Cl	*sec*-C_4H_9	O	0.0058 ± 0.0019 (3)
9	Cl	-$(CH_2)_2NH_2$	O	4.55, 5.35 (2)

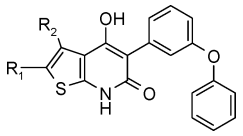
[a] Number of experiments.

more so than position 3. However, this impression was not corroborated by quantitative structure−activity relationship (QSAR) calculations: Hansch analysis on 45 compounds returned no significant term for MR (molar refractivity ≈ volume) at position 2 but a strong influence of lipophilicity (π) and electron-withdrawing potency (σ).

Our next series of compounds had chlorine or bromine in position 2, in combination with alkyl substituents of increasing size in position 3. Comparison with the previous series of compounds informed us that chloro and bromo substitution in position 2 were at least as effective as methyl and even as ethyl substitution; thus, **7a** (bromo, K_i 1.77 μM) was of similar potency as **3e** (ethyl, K_i 3.1 μM), and compounds with Br and methyl (**7g**) or with Cl and methyl (**8g**) in positions 2 and 3, respectively, were 3−5 times more potent than the dimethyl analogue **3f** ($p < 0.01$). Since chloro and methyl groups occupy roughly the same space, the higher potencies of the halogen-substituted analogues were most likely related to the higher electronegativity of the halogens. Also for quinolinone-type glycine antagonists, chloro substitution in the benzo part of the ring system is more effective than methyl substitution.[17] If the substituent in position 3 was an ethyl group, the additional introduction of Br respective to Cl into position 2 increased the potency by a factor of 50−60 (compare **7h** and **8h**, Table 3, to **3h**, Table 2). Also here, the 3-ethyl substituent could be exchanged to isopropyl or secondary butyl without loss in potency, finally returning from this series of syntheses four high-affinity inhibitors of [^{3}H]glycine binding (**7h**, **8h**, **8i**, and **8m**), with K_i values from 6 to 10 nM. And again, as in the halogen-free series shown in Table 2, 3-cyclopropyl substitution (**8k**) was not as welcome as the other alkyl substitutions ($p < 0.001$).

In this series (and in the following), we included one compound with an oxo/thio exchange at position 6 of the bicyclic ring system. The substitution pattern of this compound (**10**) was analogous to **8i**, with Cl in position 2 and isopropyl in position 3, but not so was the observed potency: **10** was more than 100 times weaker than **8i**. Thio analogues of kynurenate-type glycine antagonists have been used successfully,[18] but for thienopyridinone-type antagonists the higher electronegativity of the carbonyl oxygen seems to be of high importance. Also the second thio analogue (**20**) was 100 times weaker than the respective oxo congener (**18i**, see Table 5). In addition, we tested in the series illustrated in Table 3 the feasibility of charged substituents in position 3 as inhibitors of [^{3}H]glycine binding; however, the IC_{50} of a phenylthienopyridinone with aminoethyl in position 3 (**9**) was weaker by 3 orders of magnitude than that of analogues with uncharged residues in position 3 of comparable size (such as **8i** or **8m**). The binding of [^{3}H]raclopride to rat striatal dopamine receptors was inhibited by **9** with $IC_{50} > 10$ μM (not illustrated).

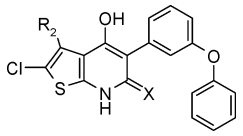
Table 4. Inhibition of [^{3}H]Glycine Binding and Cytotoxicity by 5-(3-Phenoxyphenyl)thienopyridinones: Influence of Alkyl Substitution



	R_1	R_2	[^{3}H]glycine binding K_i (μM) ± SD (n)[a]	cytotoxicity IC_{50} (μM) (n)
16a	H	H	0.368 ± 0.274 (5)	89.2 (2)
16g	H	CH_3	0.330 ± 0.172 (8)	>250
16h	H	C_2H_5	0.179 ± 0.120 (5)	>50
16l	H	CH_2OCH_3	0.036 ± 0.019 (6)	55.8 (2)
16m	H	*sec*-C_4H_9	0.096 ± 0.048 (4)	>50
16s		-$(CH_2)_3$-	0.118 ± 0.067 (6)	
16u		-$(CH_2)_4$-	0.392 ± 0.144 (4)	>30
16v		-$S(CH_2)_3$-	0.037 ± 0.009 (4)	>250
17	CH_2NH_2	CH_3	1.98 ± 1.12 (4)	

[a] Number of experiments.

Table 5. Inhibition of [^{3}H]Glycine Binding and Cytotoxicity by 5-(3-Phenoxyphenyl)thienopyridinones: Influence of Alkyl Substitution



	R	X	[^{3}H]glycine K_i (μM) ± SD (n)[a]	cytotoxicity IC_{50} (μM) (n)
18a	H	O	0.0166 ± 0.0119 (6)	15.4 (2)
18g	CH_3	O	0.023 ± 0.016 (10)	>200
18h	C_2H_5	O	0.00111 ± 0.00024 (5)	
18i	*i*-C_3H_7	O	0.00146 ± 0.00054 (5)	5.2 (2)
20	*i*-C_3H_7	S	0.139 ± 0.042 (3)	
18j	*n*-C_3H_7	O	0.00412 ± 0.00122 (4)	15.9 (2)
18k	*c*-C_3H_5	O	0.0095 ± 0.0027 (3)	
18l	CH_2OCH_3	O	0.00160 ± 0.00097 (7)	15.7 (2)
18m	*sec*-C_4H_9	O	0.0020 ± 0.0002 (3)	7.9 (2)
18n	*tert*-C_4H_9	O	0.0033 ± 0.0004 (3)	15.5 (2)
18o	*n*-C_6H_{13}	O	0.080 ± 0.043 (5)	>50
18p	*c*-C_6H_{11}	O	>30	>50
18r	CH_2OH	O	0.069 ± 0.048 (5)	
19	$(CH_2)_2NH_2$	O	16.5 ± 5.3 (3)	

[a] Number of experiments.

Also, our last series of syntheses followed the experience made with quinolinone-type glycine antagonists. Elongation of the 5-phenyl substituent by addition of a 3-phenoxy residue increased the potencies of halogen-free compounds by factors of 3−44 (Table 4) and of compounds with Cl in position 2 by factors 3−12 (Table 5). In general, compounds with affinities already in the low nanomolar range, like **8h**, **8i**, and **8m**, did not profit from this kind of substitution to the same extent as weaker compounds like **3a**, **3g**, or **8g**. Thus, no subnanomolar affinities were reached. Nevertheless, the structure/activity relationships seen without the 3′-phenoxy substitution (in Tables 2 and 3) were nicely reproduced: The highest potencies were seen with small and flexible alkyl substituents in position 3.

As in the previous series of compounds, we took the occasion to study the influence of introduction of a positively charged substituent. In the phenoxy series without Cl (Table 4), aminomethyl was introduced in position 2, together with methyl in position 3 (**17**). In comparison to the analogous compound

Table 6. Free−Wilson Analysis: Activity Contribution of Substituents[a]

		N[b]	coeff.[c]	std error[d]	factor[e]
core structure (**3a**)			4.86	0.18	
R_1	Cl	21	1.75	0.13	56.29
R_1	Br	3	1.30	0.21	19.74
R_1	C_2H_5	1	0.64	0.35	4.41
R_1	CH_3	3	0.47	0.23	2.98
R_1	CH_2NH_2	1	−0.42	0.34	−2.61
R_2	CH_2OCH_3	2	1.40	0.27	25.05
R_2	C_2H_5	5	1.38	0.20	24.26
R_2	*sec*-C_4H_9	4	1.26	0.22	18.39
R_2	*i*-C_3H_7	5	1.25	0.24	17.84
R_2	*tert*-C_4H_9	1	0.88	0.35	7.67
R_2	*n*-C_3H_7	1	0.79	0.35	6.15
R_2	*c*-C_3H_7	3	0.50	0.24	3.17
R_2	CH_3	8	0.27	0.18	1.88
R_2	CH_2OH	1	−0.44	0.35	−2.73
R_2	*n*-C_6H_{13}	1	−0.50	0.35	−3.16
R_2	$(CH_2)_2NH_2$	2	−2.55	0.28	−356.81
R_2	*c*-C_6H_{11}	1	−3.30	0.35	−1974.42
R_3	Ph-O-Ph	24	0.98	0.13	9.57
R_3	2-Th	3	−0.73	0.21	−5.35
R_1-R_2	$S(CH_2)_3$-	1	1.59	0.35	38.55
R_1-R_2	$(CH_2)_3$-	1	1.08	0.35	12.08
R_1-R_2	$(CH_2)_4$-	3	0.52	0.24	3.32
X=S		2	−1.90	0.28	−80.04

[a] For substitution scheme see Table 2. $N = 46$, $r^2 = 0.97$, $s = 0.31$, DF = 22, $F = 36.8$. [b] Frequency of occurrence. [c] Activity coefficient in log scale. [d] Standard error of coefficient. [e] Activity contribution (= exp(coeff); −exp(1/coeff) for negative values of coeff).

without that aminomethyl group (i.e., with **16g**), we observed a serious drop in potency (Table 4). Since the similarly sized ethyl has been fine in this position (see **3e**, Table 2), the positive charge (as would occur at pH 7.0) seems to be unfavorable. In the phenoxy series with Cl (Table 5), the bigger aminoethyl was introduced in position 3 (the position allowing somewhat bulkier substituents than position 2), resulting in the very weak inhibitor **19**. Again, similarly sized substituents at this position had produced inhibitors at least 3 orders of magnitude more potent, pointing once more to the handicap of a positive charge at this part of the structure. The binding of [^{3}H]raclopride to rat striatal dopamine receptors was inhibited by **19** with $IC_{50} > 30$ μM (not illustrated).

In the phenoxy compounds only, hydroxymethyl (**18r**) and methoxymethyl (**16l**, **18l**) were tested as substituents at position 3 of the thienopyridine. In the phenoxy series without Cl (Table 4), **16l** with methoxymethyl in position 3 was among the most potent compounds, interestingly only equaled by **16v** with a sulfur at position 2. In the phenoxy series with Cl (Table 5), **18l** with methoxymethyl in position 3 had the same potency as other compounds with medium-sized substituents in position 3 (**18h**, **18i**, and **18m**). It may be speculated that, in the series without Cl, the absence of the (very favorable) electronegative substituent is to some extent compensated by the presence of O or S in the substituents, whereas in the series with Cl, methoxymethyl has no additional effect. A hydrophilic substituent containing O is unfavorable as exemplified by **18r** with hydroxymethyl in position 3 (Table 5).

The results in Tables 2−5 were finally subjected to Free−Wilson (FWA) and Hansch (HA) analyses, exploring the individual contributions of functional groups at distinct positions (FWA) and the influence of molecular properties at specific sites (HA), with **3a** as the reference. The results of the FWA are shown in Table 6, and those of the HA are shown in Table 7. As already discussed, the best substituent at position 2 was chlorine, with a 56-fold better activity than hydrogen. Introduction of a H-bond active group (**17**; CH_2NH_2) decreases activity by a factor of 2.6.

Table 7. Hansch Analysis: Activity Contribution of Substituents

		coeff.	std error
core structure (**3a**)		4.44	0.20
R_1	p	1.10	0.15
R_1	σ_p	1.82	0.32
R_2[b]	MR	2.15	0.32
R_2	MR^2	−0.71	0.13
R_2	HDon	−3.66	0.33
R_3	2-Th	−0.74	0.28
R_3	Ph-O-Ph	1.19	0.14
X=S		−1.67	0.32

[a] For substitution scheme see Table 2. $N = 43$, $r^2 = 0.92$, $s = 0.42$, DF = 34, $F = 48.8$. [b] R_2: $MR_{max} = 1.47$.

At position 3 a very sound QSAR for the size of the substituent was found: the quadratic term in the HA revealed that the optimal size of a substituent was that of ethyl. Bridging both positions does not enhance activity significantly, as can be seen from the summation of the best substituents from positions 2 and 3: [R_1−R_2 = -$S(CH_2)_3$-] < (R_1 = Cl) + (R_2 = CH_2OCH_2). Elongation at position 5 from phenyl to phenoxyphenyl enhanced activity by about 1 order of magnitude (0.98 log), whereas bioisosteric substitution of phenyl by thiophene reduced activity by a factor of 5. HA returned two compounds as outliers. For **18p** (cyclohexyl in position 3), the HA most likely did not consider sufficiently its increased rigidity in comparison to the linear analogue (**18o**). For the hydroxyethyl derivative (**18r**), the HA predicted a pronounced decrease in activity, due to the H-bond donating potential of this substituent. That such a decrease did not occur to the predicted extent may have been due to internal H-bond formation with the adjacent -OH group in position 4. As already mentioned, the optimal size of a substituent in position 3 expressed as molar refractivity was MR = 1.47, very close to the value for isopropyl or *n*-propyl (MR = 1.49).

A series of these antagonists was tested in a cell toxicity assay with mouse fibroblast cell expressing human NRs. Cell death was dose-dependently inhibited by nine of the tested thienopyridinones, including the benzene analogue L-701,324 (IC_{50} for L-701,324 was 3.79 μM, with $n = 2$; value not shown in the table). The test compounds were about a thousand times more potent in the binding assay than in the cell toxicity assay, presumably due to the high (micromolar) concentration of glycine in the cell culture medium. With some compounds, no results could be obtained in the cell toxicity assay, due to direct toxicity of the compounds or to poor solubility in the incubation medium. IC_{50} values in the cell toxicity assay were well correlated to the K_i values obtained from the binding assays. For the nine compounds thath were active in both settings, a correlation coefficient $r = 0.89$ ($P = 0.001$) was observed.

A first series of compounds, **18a**, **18h**, **18i**, and **18l**, were subjected to in vivo testing in the maximal e-shock mouse model, in direct comparison with the standard compound L-701,324. L-701,324 protected against tonic extension in 50% of the mice at 0.70 mg/kg, in agreement with the literature (see ref 5). Much higher doses of **18h** (15.5 mg/kg with e-shock after 30 min, 13.5 mg/kg after 60 min) and of **18i** (43 mg/kg with e-shock after 30 min) were required to achieve the same degree of protection (Figure 1). **18a** and **18l** protected only 10% and 30%, respectively, at the highest dose tested (30 mg/kg). **18i** and L-701,324 were subsequently quantified in plasma and in brain tissue after systemic application to rats. Both of them reached much lower levels in brain tissue than in plasma

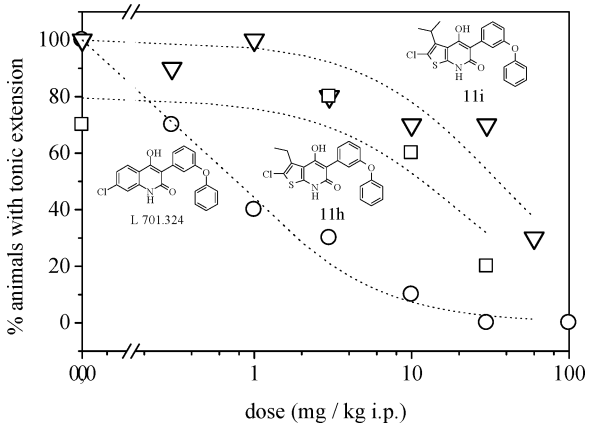


Figure 1. Protection of mice against maximal e-shock, by **18h** (□), **18i** (▽), and the reference compound **L-701,324** (○).

Table 8. Plasma and Brain Tissue Levels after Intravenous Application of **18i** and of L-701,324 to Rats

	after 6 min	after 1 h
	L-701,324 (5 mg/kg)	
plasma (μM)	125 ± 5.6 (3)	64 ± 5 (3)
brain (μmol/kg)	5.5 ± 0.5 (3)	1.4 ± 0.3 (3)
	18i (10 mg/kg)	
plasma (μM)	97 ± 21 (3)	9.7 ± 4.5 (3)
brain (μmol/kg)	3.5 ± 0.9 (3)	0.28 ± 0.13 (3)

(Table 8). Although **18i** was applied at a dose twice as high as L-701,324, its plasma and its brain tissue levels were lower than that of the reference compound, especially after the longer time delay. The weaker in vivo efficiency of **18i** as compared to L-701,324 may, thus, be a consequence of both poorer bioavailability and faster elimination.

Conclusion

In summary, a set of novel ligands with high affinity at the glycine binding site of the NR complex is presented. Optimization of the substitution pattern in the thieno part of the thieno-[2,3-*b*]pyridinone finally yielded glycine antagonists with in vitro potencies comparable to the most potent quinolinone-type antagonists known, hence confirming the chosen benzene/thiophene bioisostere approach. Our detailed survey of optimized substitution patterns may be of particular relevance since the binding pocket has been crystallized and resolved with 3.2 Å resolution.[19] Further studies will have to show whether the weak in vivo efficiency demonstrated for four of them was representative for this new class of compounds.

Experimental Section

Phenylacetylation of Thiophenes 1a−i, 1k, 1m, and 1t: General Procedure. To a solution of the appropriate aminothiophene (**1a−i**, **1k**, **1m**, or **1t**) (1 equiv) in dry dioxane was added phenylacetyl chloride (1.3 equiv), dissolved in dry dioxane, dropwise at 70 °C. After complete addition the mixture was stirred at 98 °C until the formation of HCl gas ceased. The solution was concentrated in vacuo and the residue was dissolved in DCM, washed with $NaHCO_3$ solution, dried (Na_2SO_4), and concentrated in vacuo. The resulting crude products (except **2a**, **2b**, and **2n**) were recrystallized and dried.

2-Thienylacetylation of Thiophenes 1d, 1g, and 1t: General Procedure. To a solution of the appropriate aminothiophene (**1d**, **1g**, or **1t)** (1 equiv) in dry dioxane was added 2-thienylacetyl chloride (1.3 equiv), dissolved in dry dioxane, dropwise at 70 °C. After complete addition the mixture was stirred at 98 °C until the formation of HCl gas ceased. The solution was concentrated in vacuo and the residue was dissolved in DCM, washed with $NaHCO_3$ solution, dried (Na_2SO_4), and concentrated in vacuo. The resulting crude products were crystallized by digestion in diethyl ether and recrystallized.

Halogenation Reaction: General Procedure. Compounds **2g−i**, **2k**, **2m**, **2q**, **13a**, **13q−r** (1 equiv) and NBS or NCS (1−1.9 equiv), respectively, were dissolved in dry chloroform and refluxed until no starting material was observed (TLC). The reaction mixture was diluted with DCM and extracted with water. The combined organic layers were dried (Na_2SO_4), and concentrated in vacuo, and the residue was recrystallized or purified by VFC.

Cyclization Reaction: General Procedure. (A) Method A. Compounds **2a−i**, **2k**, **2m**, **2t**, **4g**, **4h**, **5g−i**, **5k**, **5m**, **5q**, **6**, **11d**, **11g**, and **11t** (1 equiv) were dissolved in dry THF and the solution was cooled to −65 °C. $KN(Si(CH_3)_3)_2$ (0.5 M in toluene or 1 M in THF, 2.2 equiv) was added dropwise. The reaction mixture was warmed to room temperature overnight and evaporated to dryness. The residue was dissolved in water and extracted several times with diethyl ether. The aqueous layer was acidified with 2 M HCl, the precipitate was filtered by suction, washed with water, and dried. The resulting products were triturated with diethyl ether, filtered, and dried.

(B) Method B. Compounds **13a**, **13g**, **13h**, **13l**, **13m**, **13s**, **13u−w**, **14a**, **14g−r**, and **15** (1 equiv) were dissolved in dry THF and the solution was cooled to −65 °C. $KN(Si(CH_3)_3)_2$ (0.5 M in toluene or 1 M in THF; 2.2 equiv) or LDA [2 M in THF, 2.2 equiv (3.3 equiv for **13w**)] was added dropwise. The reaction mixture was warmed to 0−10 °C overnight and worked up depending on the formation of side products and the solubility of the products (for details, see Supporting Information).

3-Phenoxyphenylacetylation of Thiophenes 1a, 1g−s, and 1u−w: (A) Method A (Compounds 13a, 13q−s, 13u, and 13w). Et_3N (2.2 equiv) was added to a solution of the appropriate aminothiophene (**1a**, **1q−s**, **1u**, or **1v**; 1 equiv), 3-phenoxyphenylacetic acid (1.2 equiv), and 2-chloro-1-methylpyridiniumiodide (1.2 equiv) in dry DCM. After complete addition, the temperature was raised to reflux and the mixture was stirred at this temperature for 3 h. The mixture was diluted with DCM and washed with 6 M HCl (except **13q** and **13w**, which were extracted with water) and $NaHCO_3$ solution, dried (Na_2SO_4), and concentrated in vacuo. The resulting crude products were purified by VFC and dried.

(B) Method B (Compounds 13g−i and 13p). 3-Phenoxyphenylacetic acid (1.3 equiv) was dissolved in dry dioxane, $SOCl_2$ (1.95 equiv) was added, and the mixture was heated to 78 °C for 3 h. The excess $SOCl_2$ was removed in vacuo, and the residue was diluted with dry dioxane and added dropwise to a dry dioxane solution of the appropriate aminothiophene (**1g−i**, **1p)** (1 equiv) at 70 °C. After complete addition, the temperature was raised to 98 °C and the mixture was stirred at this temperature until the formation of HCl gas ceased. The solution was concentrated in vacuo and the residue was dissolved in DCM, washed with $NaHCO_3$ solution, dried, and concentrated in vacuo. The resulting crude products were purified by VFC and dried.

(C) Method C (Compounds 13j−o and 13v). To a solution of the appropriate aminothiophene (**1j−o** or **1v**) (1 equiv) in dry dioxane was added 3-phenoxyphenylacetylchlorid[20] (1.1 equiv), dissolved in dry dioxane, dropwise at 70 °C. The further procedure was as described for the preceding Method B.

Radioligand Binding Assays. The affinities of the test compounds for the glycine regulatory site of the NMDA receptor complex were evaluated by analyzing their abilities to displace [^{3}H]-glycine in the presence of 10 μM glutamic acid from rat hippocampal membranes. We used 4−5 different inhibitor concentrations inducing 20−90% inhibition of specific binding. In parallel, the *apparent* affinity of [^{3}H]glycine was evaluated in each experiment and used to calculate the concentration of unavoidable background glycine, assuming a *true* affinity constant of 40 nM.[21] This strategy allowed the reliable calculation of K_i values from the experimentally obtained IC_{50} values. The ethylamines **9** and **19** were, in addition, tested as inhibitors of specific [^{3}H]raclopride binding[22] to rat striatal membranes. To estimate the significance of differences of various K_i values, they were comprised into groups of 3−5 values as

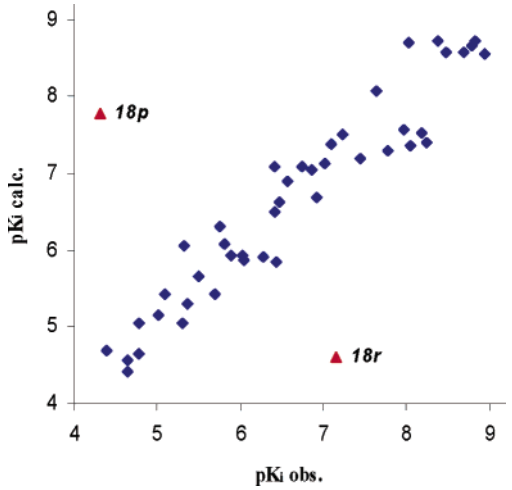


Figure 2. Correlation of calculated versus observed activities from the Hansch analysis. Activities are shown as pK_i values [= −log (1/K_i)]. The labeled compounds (**18p** and **18r**) have been excluded from model generation.

appropriate for the respective argument raised in the discussion and subjected to statistical analysis (ANOVA with post hoc Newman−Keuls test).

QSAR. To gain insight into the quantitative structure−activity relationship (QSAR) of the compounds synthesized, two kind of analyses were performed. First, the individual contribution of a functional group at a distinct position was evaluated by means of a Free−Wilson analysis[23] (FWA). For this approach compound **3a** was chosen as reference. In a table for each substituent at the positions 2, 3, and 5 a column was built as well as for annulated rings in position 2 and 3 and sulfur at position 6 (**10**, **20**). All compounds were stored as rows with their activity expressed as pK_i (Figure 2). The resulting Free−Wilson matrix was solved by conventional multiple linear regression approaches.[24] For Hansch analysis[25] (HA), the substituent parameters MR (molar refractivity), π (lipophilicity), σ (Hammett constant), and L, B_1, B_5 (Sterimol) were assigned for each group in positions 2 and 3; one compound, **16v**, could not be included because of missing descriptors. Substitution pattern at positions 5 and 6 were encoded as indicator variables such as at the FWA. As a quality measure of both methods, the standard error and the Fischer test variable were calculated.

Cell Culture. Mouse fibroblast L(tk$^-$) cells were cotransfected with the dexamethasone-inducible mammalian transfection vectors pMSG-NR1a and pMSG-NR2A containing the human cDNAs coding for NR1a or NR2A as described elsewhere.[11] In these cell lines, induction of NR expression in the transformed L(tk$^-$) cells leads to cell death caused by Ca^{2+} overload. For in vitro testing of compounds, the clone L12-G10 showing the highest rate of cell death and inhibition by antagonists was selected. To demonstrate the in vitro activity of compounds, 15×10^3 cells/well were seeded in a 96-well plate and grown overnight in MEM containing 10% heat-inactivated fetal calf serum (FCS), 2 mM Glutamax I, 100 units/mL penicillin, 100 μg/mL streptomycin, and 0.5 mM sodium pyruvate at 5% CO_2. Receptor expression was induced by replacing growth medium with induction medium (MEM without Mg^{2+}/phenol red containing 5% FCS, 50 mM KCl, 2 mM Glutamax I, 100 units/mL ampicillin, 100 μg/mL streptomycin, and 0.5 mM sodium pyruvate and 4 μM dexamethasone). As FCS contains saturating concentrations of glutamate and glycine, supplementation of agonists was not necessary. To avoid variations in agonist concentration, always the same FCS batch was used. The cells were incubated with increasing concentrations of compounds for 24 h.

Cell Toxicity Assay. Cell death rate was measured by detection of lactate dehydrogenase (LDH) release into culture supernatants. Supernatant (50 μL) was incubated with LDH assay mixture in 96-well plates (pretreated with 1% bovine serum albumin, BSA) for 20 min at 25 °C. LDH assay mixture contained 1% sodium L-lactate in Tris, pH 9.0, 1.4 mM NAD^+, 20 mM phosphate buffer, pH 7.4, 0.8 mg of BSA, 18 units of diaphorase, and 4 mg of INT per milliliter. The coupled enzymatic reaction results in the conversion of iodonitrotetrazolium salt (INT) into a red formazan product. After 20 min the reaction was stopped by addition of 50 μL of 1 M acetic acid and absorption was measured at 490 nm in a Dynatech MR 5000 Elisa-reader. The intensity of the red color formed is directly proportional to the number of lysed cells. Cytosolic LDH of surviving cells was released into blank medium containing 0.1% Triton X-100 and measured as mentioned above. Data were corrected for cell culture medium background. Cell death rate (CD) was calculated as follows: CD (%) = $A_{LDHrel}/(A_{LDHrel} + A_{LDHcyt}) \times 100$. Obtained data were fitted to a four-parameter logistic function by use of Sigma Plot. To prevent measurement of nonspecific toxicity, the applicable concentration range of each compound was determined by trypan blue exclusion with non-dexamethasone-treated cells. After 24 h incubation, 0.04% trypan blue in phosphate-buffered saline (PBS) was added and dye exclusion was checked by light microscopy. Only nontoxic inhibitor concentrations were used.

Mouse Seizure Model. Ten male Swiss mice per dosage received intraperitoneally six doses of L-701,324 (0.3−100 mg/kg); three doses of **18a**, **18h**, and **18l** (3−30 mg/kg); six doses of **18i** (0.3−60 mg/kg); or vehicle (8 parts DMSO, 10 parts H_2O). Maximal e-shock was applied 30 or 60 min later, and tonic hind limb extension was rated as an unprotected response. Dose/response data were fitted to the function: response (dose = x) = response (dose = 0) $\times$ $ID_{50}/(ID_{50} + x)$, where x is the dose of the compound tested, and ID_{50} the dose inhibiting the response in 50% of the animals.

Drug Concentrations in Blood and Brain of Rats. In six male Wistar rats, plasma and brain tissue levels of **18i**, one of our most potent compounds, were measured 6 min (n = 3) and 1 h (n = 3) after intravenous administration of a dose of 10 mg/kg. In another six rats, similar measurements were performed after application of 5 mg/kg of the reference glycine antagonist L-701,324. Both compounds were quantified by an analytical HPLC technique.

Supporting Information Available: Experimental details of all intermediates and target compounds with supporting spectral data and C, H, N analysis results. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Cotman, C. W.; Kahle, J. S.; Miller, S. E.; Ulas, J.; Bridges, R. J. Excitatory amino acid neurotransmission. In *Psychopharmacology: The fourth Generation of Progress*; Bloom, F. E., Kupfer D. J., Eds.; Raven Press: New York, 1995; pp 75−85.
(2) Malenka, R. C.; Nicoll, R. A. NMDA-receptor-dependent synaptic plasticity: multiple forms and mechanisms. *Trends Neurosci.* **1993**, *16*, 521−527.
(3) Meldrum, B.; Garthwaite J. Excitatory amino acid neurotoxicity and neurodegenerative disease. *Trends Pharmacol. Sci.* **1990**, *11*, 379−387.
(4) Kleckner, N. W.; Dingledine R. Requirement for glycine in activation of NMDA-receptors expressed in *Xenopus* oocytes. *Science* **1988**, *241*, 835−837.
(5) Leeson, P. D.; Iversen L. L. The glycine site on the NMDA receptor: Structure−activity relationships and therapeutic potential. *J. Med. Chem.* **1994**, *37*, 4053−4067.
(6) Jansen, M.; Dannhardt, G. Antagonists and agonists at the glycine site of the NMDA receptor for therapeutic interventions. *Eur. J. Med. Chem.* **2003**, *38*, 661−670.
(7) Johnson, J. W.; Ascher, P. Glycine potentiates the NMDA response in cultured mouse brain neurons. *Nature* **1987**, *325*, 529−531.
(8) Kemp, J. A.; Foster, A. C.; Leeson, P. D.; Priestley, T.; Tridgett, R.; Iversen, L. L.; Woodruff, G. N. 7-Chlorokynurenic acid is a selective antagonist at the glycine modulatory site of the NMDA receptor complex. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 6547−6550.
(9) Kleckner, N. W.; Dingledine, R. Selectivity of quinoxalines and kynurenines as antagonists of the glycine site on NMDA receptors. *Mol. Pharmacol.* **1989**, *36*, 430−436.

(10) McQuaid, L. A.; Smith, E. C. R.; Lodge, D.; Pralong, E.; Wikel, J. H.; Calligaro, D. O.; O'Malley, P. J. 3-Phenyl-4-hydroxyquinolin-2(1*H*)-ones: Potent and selective antagonists at the strychnine-insensitive glycine site on the NMDA receptor complex. *J. Med. Chem.* **1992**, *35*, 3423−3425.
(11) Steinmetz, R. D.; Fava, E.; Nicotera, P.; Steinhilber, D. A simple cell line based in vitro test system for *N*-methyl-D-aspartate (NMDA) receptor ligands. *J. Neurosci. Methods* **2002**, *113*, 99−110.
(12) Gottschlich, R.; Leibrock, J.; Noe, C. R.; Berger, M. L.; Buchstaller, H.-P. Thienopyridones. European Patent EP-717044, 1996.
(13) (a) Gewald, K. 2-Amino-thiophen aus α-Oxo-mercaptanen und methylenaktiven Nitrilen. *Chem. Ber.* **1965**, *98*, 1. (b) Gewald, K.; Schinke, E.; Böttcher, H. 2-Amino-thiophene aus methylenaktiven Nitrilen, Carbonylverbindungen und Schwefel. *Chem. Ber.* **1966**, *99*, 94. (c) Gewald, K.; Schinke, E. Notiz zur Reaktion von Aceton mit Cyanessigester und Schwefel. *Chem. Ber.* **1966**, *99*, 2712.
(14) Binder, D.; Hillebrand, F.; Noe, C. R. Synthesis of substituted thieno-[2,3-*d*]imidazoles. *J. Chem. Res. (M)* **1979**, 1151−1163.
(15) (a) Buchstaller, H.-P.; Siebert C. D.; Lyssy, R. H.; Ecker, G.; Krug, M.; Berger, M. L.; Gottschlich, R.; Noe. C. R. Thieno[2,3-*b*]-pyridinones as Antagonists on the Glycine Site of the *N*-methyl-D-aspartate Receptor − Binding Studies, Molecular Modeling and Structure−Activity-Relationships. *Sci. Pharm.* **2000**, *68*, 3−14. (b) Buchstaller, H.-P.; Siebert, C. D.; Lyssy, R. H.; Frank, I.; Duran, A.; Gottschlich, R.; Noe, C. R. Synthesis on Novel 2-Aminothiophene-3-carboxylates by Variations of the *Gewald* Reaction, *Monatsh. Chem.* **2001**, *132*, 279−293. (c) Noe, C. R.; Buchstaller, H.-P.; Siebert C. D. Synthese von 4-Alkyl-2-amino-3-thiophencarbonsäureestern. *Pharmazie* **1996**, *51*, 833−836.
(16) Kappe, T.; Karem, A. S.; Stadlbauer, W. Synthesis of benzo-halogenated-4-hydroxy-2(1*H*)-quinolones. *J. Heterocycl. Chem.* **1988**, *25*, 857−62.
(17) Rowley, M.; Leeson, P.; Grimwood, S.; Marshall, G.; Saywell, K. 5,6,7,8-Tetrahydroquinolones as antagonists at the glycine site of the NMDA receptor. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 2089−2092.
(18) Moroni, F.; Alesiani, M.; Galli, A.; Mori, F.; Pecorari, R.; Carla, V.; Cherici, G.; Pellicciari, R. Thiokynurenates: a new group of antagonists of the glycine modulatory site of the NMDA receptor. *Eur. J. Pharmacol.* **1991**, *199*, 227−232.
(19) Furukawa, H.; Gouaux, E. Mechanisms of activation, inhibition and specificity: crystal structure of the NMDA receptor NR1 ligand-binding core. *EMBO J.* **2003**, *22*, 2873−2885.
(20) Matthies, D.; Buechling, U. Cyclopropanone hemiamidals: synthesis and application in amidoalkylations. *Arch. Pharm.* **1983**, *316*, 598−608.
(21) Berger, M. L. On the true affinity of glycine for its binding site at the NMDA receptor complex. *J. Pharmacol. Toxicol. Methods* **1995**, *34*, 79−88.
(22) Dewar, K. M.; Montreuil, B.; Grondin, L.; Reader, T. A. Dopamine D_2 receptors labeled with [^{3}H]raclopride in rat and rabbit brains. Equilibrium binding, kinetics, distribution and selectivity. *J. Pharmacol. Exp. Ther.* **1989**, *250*, 696−706.
(23) Kubinyi, H. Free Wilson Analysis. Theory, Applications and its Relationship to Hansch Analysis. *Quant. Struct.-Act. Relat.* **1988**, *7*, 122−133.
(24) Cornerstone, version 3.7.1, Brooks Automation, Inc. Chelmsford, MA.
(25) Hansch, C.; Leo, A. *Exploring QSAR, Fundamentals and Applications in Chemistry and Biology*; American Chemical Society: Washington, DC, 1995.

JM0503493