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Synthesis and anticonvulsant evaluation of some new 2-substituted-3-arylpyrido[2,3-*d*]pyrimidinones

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Abstract—A series of 2-substituted-3-arylpyrido[2,3-*d*]pyrimidinones was prepared for evaluation as potential anticonvulsants. In murine screening, compounds **4a–c** having a 2-oxo-2-(4-pyridyl)ethyl group in the 2-position and a 2-substituted phenyl moiety at the 3-position of the pyridopyrimidinone system displayed the most potent anti-seizure activity in both the maximal electroshock (MES) and pentylenetetrazol (scPTZ) tests at doses in the 3–10 mg/kg range. Compound **4c** showed no agonist activity at the $GABA_A$ receptor and was unable to block presynaptic sodium and calcium channels in vitro.

1. Introduction

For more than three decades, interest in 3*H*-quinazolin-4-ones as potential sedative-hypnotic and/or anticonvulsant drugs has led to the preparation and pharmacological evaluation of literally hundreds of such molecules.[1] In spite of this effort, only one compound, 2-methyl-3-(2-methylphenyl)-3*H*-quinazolin-4-one (methaqualone) **1**,[2] emerged as a clinically adopted, sedative-hypnotic drug. However, marketing and clinical use of **1** was terminated in 1984 when it was transferred to Schedule I of the Controlled Substances Act because of its abuse liability.[3] This, coupled with the tendency of **1** and related 3H-quinazolin-4-ones to present sedative–hypnotic (neurotoxic) TD_{50}s similar to their ED_{50}s for anticonvulsant activity in traditional murine models has been a major deterrent to the development of these compounds as possible antiepileptic drugs (AEDs).

In a previous study,[4] we discovered that several 2-substituted-3-aryl-3*H*-quinazolin-4-ones of structural type **2**[5] possessed good activity against pentylenetetrazol (scPTZ)-induced seizures in mice (ip) with relatively low neurotoxicity. The 2-oxo-2-(4-pyridyl) ethyl group at the 2-position of the quinazolin-4-one ring system in conjunction with a 2-substituted phenyl moiety at the 3-position were identified as key functional groups (Fig. 1 and **2a–c**). Quinazolinone **2d**, which lacks a 2-substituent in the 3-aryl ring was active only at doses of 300 mg/kg, which was 10 times less effective at controlling scPTZ-induced seizures than **2a–c**; **2d** was completely inactive against seizures induced by maximal electroshock.[4] Unfortunately, this promising separation of anticonvulsant and neurotoxic properties observed in the ip scPTZ mice screens of compounds **2a–b** with protective index values (PIs) of 5.6 and 18, respectively, was negated in oral scPTZ assessment in rats (PIs of 1.1 and 0.94, respectively).[4] An earlier study by Blanton and co-workers,[6] describing that 2-methyl-3-(2-methylphenyl)pyrido[2,3-*d*]-4(3*H*)-pyrimidinone (**3a**) possessed anti-scPTZ activity comparable to methaqualone (**1**), but with lower neurotoxicity, prompted us to undertake the preparation of a series of new pyrido[2,3-*d*]pyrimidinones **4** in the hope of achieving a more favorable separation of anticonvulsant and sedative characteristics than displayed by our former quinazolinone counterparts **2**. In addition, we were also interested in preparing selected 2-(2-arylethenyl)pyrido[2,3-*d*]pyrimidinones **5** in light of the reported antiseizure activity of similarly substituted 3*H*-quinazolin-4-ones.[1a] A number of

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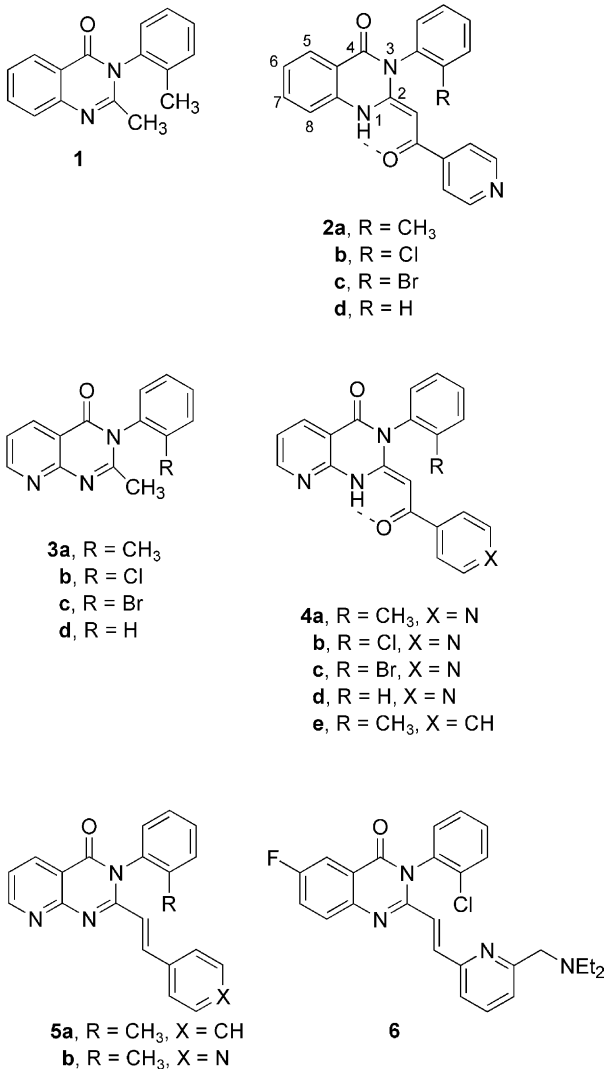


Figure 1.

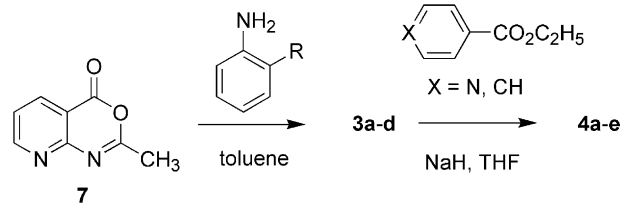
2-(2-arylethenyl)quinazolinones structurally related to **5** have been prepared recently and were shown to be effective noncompetitive antagonists of the excitatory α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor.[7] In this same study, racemate **6** was found to be a potent blocker of scPTZ-induced seizures in mice (ED_{50} = 3.8 mg/kg).[8]

Against this background, we now wish to describe the synthesis of compounds **3**–**5** and the results of their pharmacological evaluation as possible anticonvulsant agents.

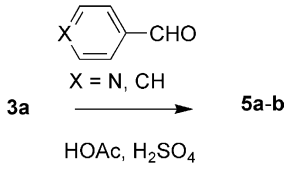
2. Results and discussion

2.1. Synthesis

2-Methyl-3-arylpyridopyrimidinones **3a**–**d** were prepared from 2-methyl-pyrido[2,3-*d*][1,3]oxazin-4-one (**7**)[9] and the appropriate aromatic amine in refluxing toluene solution. Acylation of **3a**–**d** was accomplished by the addition of sodium hydride in the presence of the required aromatic ester[4] to afford 2-(2-oxoalkyl) derivatives **4a**–**e** (Scheme 1). Acid-catalyzed condensation of **3a** with benzaldehyde and 4-pyridinecarboxaldehyde gave excellent yields of compounds **5a** and **5b**, respectively (Scheme 2).



Scheme 1.



Scheme 2.

2.2. Pharmacological evaluation

Pharmacological testing of pyridopyrimidinones **3**–**5** was performed by the Epilepsy Section of the National Institute of Neurological Disorders and Stroke (NINDS) using standard protocol adopted by the Anti-epileptic Drug Development (ADD) program.[10] Anticonvulsant activity was evaluated in the maximal electroshock seizure (MES) and subcutaneous pentylenetetrazol (scPTZ) tests; neurological impairment (TD_{50}) was assessed by the rotorod test (Table 1).

Preliminary screening data for 2-methyl-3-arylpyridopyrimidinones **3a**–**d** (Table 1) indicated that compounds **3a**–**c** possessing a 2-substituted phenyl moiety at the 3-position of the pyridopyrimidinone ring system exhibited modest activity at 100 mg/kg against both MES- and scPTZ-induced seizures, while 3-phenyl-substituted compound **3d** was active only in the scPTZ test. All four compounds caused rotorod toxicity at the 100 mg/kg dosage level and therefore showed no separation between anticonvulsant activity and neurotoxicity.

Introduction of the 2-oxo-2-(4-pyridyl)ethyl group at the 2-methyl position of 2-substituted phenyl compounds **3a**–**c** to afford **4a**–**c** resulted in as much as a 30-fold increase in anticonvulsant potency against seizures induced by both MES and scPTZ when compared to their precursors **3a**–**c** (Table 1). Similar incorporation of this group into 3-phenyl-substituted compound **3d** caused an increase in the MES activity for the resulting **4d**. On the other hand, no enhancement in anticonvulsant activity was realized when the 2-methyl group of **3a** was replaced by the analogous 2-oxo-2-phenethyl moiety, cf. compound **4e**. This activity trend, which we

Table 1. Pharmacological activity of pyrido[2,3-*d*]pyrimidinones **3**–**5**[a]

Compd	MES[b,e]		sc PTZ[c,e]		Toxicity[d,e]	
	0.5 h	4.0 h	0.5 h	4.0 h	0.5 h	4.0 h
3a	++(2/3)	+(1/1)	++(4/5)	+(1/1)	+++(1/4)	+(2/2)
3b	++(3/3)	++(2/3)	++(1/1)	++(5/5)	++(8/8)	++(2/4)
3c	++(1/1)[f]	—	++++(1/5)	++(3/5)	++(4/4)	—
3d	—	—	++(3/5)	—	++(3/8)	—
4a	+++++(2/4)	+++(1/1)	+++++(1/4)	+++(1/1)	++++(6/8)	+++(2/2)
4b	+++++(2/4)	+++(1/1)	+++++(2/4)	+++(1/1)	++++(5/8)	+++(2/2)
4c	++++(3/4)	+++(1/1)	+(1/1)	++++(1/4)	++++(2/4)	+++(2/2)
4d	++(1/3)	++(3/3)	++(4/5)	++(5/5)	++(1/8)	++(4/4)
4e	—	+(1/1)	—	+(2/5)	++(1/8)	++(1/4)
5a	+(1/1)	++(1/3)	+(3/5)	—	++(2/8)	++(2/4)
5b	+(1/1)	—	—	—	+(1/4)	+(1/2)

[a] The test compounds were administered ip to mice.
[b] Maximal electroshock seizure test (number of animals protected/number of animals tested).
[c] Subcutaneous pentylenetetrazol test.
[d] Neurologic toxicity (number of animals exhibiting toxicity/number of animals tested).
[e] Activity and toxicity at 3, 10, 30, 100, and 300 mg/kg are indicated by +++++, ++++, +++, ++, and +, respectively; — denotes no antiseizure or toxicity observed up to 300 mg/kg.
[f] 2/3 animals were protected at 30 mg/kg after 0.25 h.

initially observed in the analogous quinazolinone series,[4] again confirms the pharmacophoric nature of this pyridyl containing moiety when combined with a 2-substituted phenyl substituent at position 3. The most active compounds, **4a** (R = CH_3) and **4b** (R = Cl), showed anti-MES and anti-scPTZ activity at doses of 3 mg/kg in preliminary screening, while **4c** (R = Br) was active in both tests at 10 mg/kg. However, all three compounds also displayed neurological toxicity at dosage levels of 10 mg/kg.

In light of the similar pharmacological profiles of **4a**–**b**, it may be concluded that the electron releasing ($-\sigma$) versus electron withdrawing ($+\sigma$) nature of the 3-phenyl substituent (R = CH_3 or Cl) has little effect on anticonvulsant potency. Rather, as observed previously in the analogous quinazolinone series,[4] the lipophilicity ($+\pi$) and position of substitution appear to be the important factors for determining antiseizure efficacy.

On the basis of their preliminary anticonvulsant potential, compounds **4b** and **4c** were selected for quantitative evaluation of their pharmacological parameters as shown in Table 2, where data for compounds **1**, **2a**–**c**, **3c** and phenobarbital are also included for comparison. Median effective doses (ED_{50}s) and median neurotoxic doses (TD_{50}s) were determined at the time of peak anticonvulsant and neurotoxic effect. The mouse ip data for **4c** corroborate the preliminary findings that this compound is a powerful antiseizure agent in both the MES (ED_{50} = 6.82 mg/kg) and scPTZ (ED_{50} = 6.98 mg/kg) assays and that it is much more potent in both screens than its corresponding unelaborated 3-methyl analog, **3c**, which exhibited ED_{50}s of 60.3 mg/kg and 30.9 mg/kg, respectively. However, as a consequence of its neurotoxicity potency (TD_{50} = 17.37 mg/kg), **4c** has marginal protective index (PI) values of ca. 2.5 in both tests. Comparison of the ED_{50} values of ip administered **4c** with quinazolinones **2a**–**c** in the same murine model reveals that **4c** is more potent in both MES and scPTZ tests.

As illustrated in Table 2, compound **4c** and its 2-chlorophenyl analog, **4b**, exhibited similar pharmacological profiles following po administration in rats, that is, each displayed excellent antiseizure activity in the MES test with nearly identical ED_{50} values of 2.56 and 2.49 mg/kg, and similar TD_{50} values of 8.61 and 8.86 mg/kg, respectively. A comparison of the ED_{50} values of **4c** and **2a**–**c** from rat po testing reveals that these compounds all have similar potencies against MES-induced seizures while **4c** is ca. 3 times less effective in controlling seizures caused by scPTZ. These results contrast with those obtained in mice ip testing as noted previously, which indicated that **4c** was much more potent than **2a**–**c** in both tests.

Replacement of the methyl group at the 2-position of **3b** with an arylethenyl group to afford pyridopyrimidinones **5a**–**b** led to a general decrease in anticonvulsant potency relative to **3b**, with styryl-substituted compound **5a** exhibiting the greater activity and neurotoxicity (Table 1). These results stand in contrast to the reported enhancement of MES activity observed by Boltze[1] following similar alterations of methaqualone (**1**).

Finally, although **4a**–**e** and **5a**–**b** like compound **6** probably exist as racemates of potentially resolvable atropisomers,[8] their resolution was not attempted because of the modest antiseizure activity exhibited by **4d**–**e** and **5a**–**b** (100–300 mg/kg) and marginal separation of anticonvulsant activity and neurotoxicity in **4a**–**c**.

As part of the present study we explored possible mechanisms by which the newly synthesized pyridopyrimidinones **4** as well as their quinazolinone analogs **2** might be exerting their anticonvulsant action. Our earlier observation[4] that quinazolinone **2b** provided protection against picrotoxin-induced seizures in mice suggested a possible mechanism of anticonvulsant action for this class of compounds, and perhaps for the structurally similar pyridopyrimidinones **4**, involving agonist activity at $GABA_A$ receptors.

Table 2. Quantification of anticonvulsant activity and neurotoxicity

Compd	Test type	ED_{50}[a,b] mg/kg		TD_{50}[b,c] mg/kg	PI[d]	
		MES	scPTZ		MES	scPTZ
1[e]	ip (mice)	52 (48–56)	33.5 (28–40)	55 (47–65)	1.06	1.64
2a[f]	ip (mice)	148[g] (112–196)	100[g] (56–197)	561[g] (425–733)	3.8	5.6
2a[f]	po (rat)	1.73[i] (1.27–2.21)	2.57[i] (2.00–3.10)	2.8[i] (2.36–3.54)	1.7	1.1
2b[f]	ip (mice)	196[g] (155–240)	14.5[g] (5.6–24.3)	262[g] (125–400)	1.3	18
2b[f]	po (rat)	1.44[i] (1.17–1.73)	3.09[i] (2.41–3.81)	2.91[i] (2.48–3.40)	2.0	0.94
2c	ip (mice)	21.8[j] (17.8–26.9)	16.0[j] (9.6–30.0)	31.5[j] (26.0–43.1)	1.44	1.97
2c	po (rat)	4.13[g] (2.83–5.01)	2.66[g] (1.48–4.27)	3.87[i] (2.59–5.01)	0.94	1.45
3c	ip (mice)	60.3[h] (44.6–77.2)	30.9[j] (22.0–42.8)	49.4[i] (46.6–52.8)	0.82	1.60
4c	ip (mice)	6.82[j] (5.85–8.27)	6.98[h] (5.39–8.57)	17.4[j] (14.3–21.0)	2.54	2.49
4c	po (rat)	2.56[g] (1.45–4.09)	<8.60[i] —	8.61[k] (5.70–12.9)	3.36	<1.00
4b	po (rat)	2.49[k] (1.78–3.22)	>31[i] —	8.86[h] (6.90–12.1)	3.55	<0.29
PB[l]	ip (mice)	20.1 (14.8–31.6)	12.6 (7.99–19.1)	96.8 (79.9–115)	4.82	7.69
PB	po (rat)	9.14 (7.58–11.9)	11.6 (7.74–15.0)	61.1 (43.7–95.8)	6.68	5.29

[a] Determined at the time of peak effect (TPE).
[b] Values in parentheses are 95% confidence intervals determined by probit analysis.
[c] Determined at the time of peak neurologic deficit (rotorod test).
[d] Protective index (TD_{50}/ED_{50}).
[e] See Ref. 6.
[f] See Ref. 4.
[g] TPE = 2 h.
[h] TPE = 0.50 h.
[i] TPE = 4 h.
[j] TPE = 1 h.
[k] TPE = 0.25 h.
[l] PB = phenobarbital.

Using a modification of an established assay[11] for measuring radioactive chloride flux at the $GABA_A$ receptor, quinazolinones **2a**–**b** were found to activate ^{36}Cl uptake, with compound **2b** ($EC_{50} = 48 \pm 2.1\,\mu M$) being more than twice as potent as **2a** ($EC_{50} > 100\,\mu M$).[12] Confirmation that this activation resulted from actions on the $GABA_A$ receptor was obtained from the observation that chloride uptake stimulated by **2b** was completely blocked by the $GABA_A$ receptor antagonists picrotoxinin and bicuculline at concentrations of 100 and 30 μM, respectively.

Similar screening of pyridopyrimidinone **4c** for $GABA_A$ receptor activation revealed that this compound was not effective in increasing the level of chloride uptake. On the contrary, preincubation of **4c** showed it to be an *antagonist* of GABA-stimulated chloride transport having an IC_{50} value of $28 \pm 1.3\,\mu M$. In addition, dose–response studies with GABA demonstrated that the primary effect of **4c** (10 μM) was to suppress the maximal extent of GABA-dependent chloride uptake, which was decreased 36% compared to GABA alone.

Additional experiments were performed in order to identify other possible target sites of action for **4c**. We tested the ability of **4c** to block 30 μM veratridine stimulated release of preloaded [^{3}H]serotonin from mouse cortical synaptosomes,[13] which would indicate possible involvement of voltage-sensitive sodium and calcium channels in the action of this compound. However, when applied at a concentration of 100 μM, **4c** had no effect on veratridine-stimulated release of labeled serotonin.

3. Conclusions

Of the pyrido[2,3-*d*]pyrimidinones prepared and screened in this study, compounds **4a**–**c** containing the pharmacophoric 2-[-2-oxo-2-(4-pyridyl)ethyl] group and a 2-substituted 3-aryl moiety exhibited potent anti-MES and anti-scPTZ activity in murine models. Following ip administration in mice, these compounds were significantly more potent than their 4(3*H*)-quinazolinone counterparts **2a**–**c** for sei-

zure control, but were also considerably more neurotoxic.

In spite of their enhanced anticonvulsant potency relative to **2a**–**c**, compounds of structural type **4** did not show the separation of anticonvulsant and neurotoxic activity we hoped for when this study was initiated. Although the MES and scPTZ ED_{50} values of **4c** compare very favorably with those of the prototype drug phenobarbital (Table 2), the greater neurotoxicity of **4c** results in a much less attractive margin of protection.

In in vitro testing, quinazolinones **2a** and **2b** were shown to be moderate agonists at the $GABA_A$ receptor, with **2b** being more than twice as effective as an agonist compared to **2a**. This difference is reflected in their relative effectiveness for suppressing picrotoxin-induced seizures in vivo; ED_{50} = 153 mg/kg for **2b**, while **2a** was ineffective in doses up to 600 mg/kg.[4] This correlation supports the premise that effects on the $GABA_A$ receptor may underlie at least some of the anticonvulsant effects of these compounds.

In contrast to **2a** and **2b**, pyridopyrimidinone **4c** showed no $GABA_A$ agonist activity. Compound **4c** also failed to block presynaptic sodium and calcium channels. This was surprising in light of its structural similarity to **2a**–**b** and its potency against scPTZ-induced seizures. Perhaps, like **2b** and **6**, the anticonvulsant action of **4c** may be directed at a different target, such as the AMPA receptor.[7,14] However, in light of its considerable neurotoxicity, which severely limits the potential of **4c** as a viable anticonvulsant drug candidate, further in vitro studies were not performed. The antagonism of GABA receptor function by **4c** was similar in potency to that of picrotoxinin (IC_{50} = 11 μM) in chloride flux assays.[11] This effect may play a role in the neurotoxicity observed with compound **4c** in whole animal screens.

4. Experimental

4.1. General

Melting points were determined on a Thomas–Hoover melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded using either a Bruker WP 270 (270 MHz) or an AM 360 (360 MHz) instrument. Combustion analyses were performed by Atlantic Microlab, Inc., Norcross, GA. Flash chromatography was carried out using 230–400 mesh Merck Kieselgel 60 silica gel. Tetrahydrofuran (THF) was dried by distillation from a solution of potassium/benzophenone ketyl. 2-Methyl-pyrido[2,3-*d*][1,3]oxazin-4-one (**7**) was obtained in 54% overall yield in four steps from quinolinic acid by a modification of the procedure of Preuss and Hoffman.[9] 2-Styryl-3-(2-methylphenyl)pyrido[2,3-*d*]-4(3*H*)-pyrimidinone (**5a**) was prepared by acid-catalyzed condensation of **3a** with benzaldehyde in 94% yield using the procedure of Kassem and Soliman.[15]

4.2. General procedure for the preparation of 2-methyl-3-arylpyrido[2,3-*d*]-4(3*H*)-pyrimidinones

To a 250 mL three-neck round bottom flask equipped with a Dean–Stark trap was added 2-methyl-pyrido[2,3-*d*][1,3]oxazin-4-one (**7**) (4.86 g, 30.0 mmol), 2-bromoaniline (5.16 g, 30 mmol) and toluene (150 mL). As the mixture was heated under nitrogen, solution was achieved. Water was azeotroped off as the solution was heated under reflux for 8 h. Upon cooling, 2-acetamidonicotinic acid (1.30 g) precipitated, which was filtered and washed with toluene, mp 189–190 °C (lit.[1b] mp 184–188 °C). The orange mother liquor was concentrated to ca. 50 mL and 6.48 g (68%) of 2-methyl-3-(2-bromophenyl)pyrido[2,3-*d*]-4(3*H*)-pyrimidinone (**3c**) crystallized, mp 200–202 °C. Recrystallization from AcOEt gave **3c** as pale-yellow crystals, mp 204–205 °C. ^{1}H NMR (270 MHz, $CDCl_3$) δ 2.31 (s, 3H), 7.45 (m, 3H), 7.55 (m, 1H), 7.82 (dd, 1H, J = 1.4, 6.0 Hz) 8.61 (dd, 1H, J = 2.0, 7.9 Hz), 9.01 (dd, 1H, J = 2.0, 4.6 Hz). ^{13}C NMR (360 MHz, $CDCl_3$) δ 24.0, 115.9, 122.3, 122.6, 129.3, 129.7, 131.3, 134.1, 136.5, 136.8, 156.3, 157.6, 157.7, 161.7. Anal. Calcd for $C_{14}H_{10}N_3OBr$: C, 53.15; H, 3.19; N, 13.29. Found: C, 53.01; H, 3.38; N, 13.26.

4.3. 2-Methyl-3-phenylpyrido[2,3-*d*]-4(3*H*)-pyrimidinone (3d)

From (**7**) (14.23 g, 87.8 mmol) and aniline (8.15 g, 88 mmol) was obtained 10.9 g (52%) of **3d**[16] mp 203–204 °C. ^{1}H NMR (270 MHz, $CDCl_3$) δ 2.31 (s, 3H), 7.39 (m, 5H), 7.56 (dd, 1H, J = 1.6, 6.2 Hz), 8.55 (dd, 1H, J = 2.0, 7.8 Hz), 8.97 (dd, 1H, J = 2.0, 4.6 Hz). Anal. Calcd for $C_{15}H_{13}N_3O$: C, 71.70; H, 5.21; N, 16.72. Found: C, 71.47; H, 5.29; N, 16.58.

4.4. 2-Methyl-3-(2-methylphenyl)pyrido[2,3-*d*]-4(3*H*)-pyrimidinone (3a)

From (**7**) (9.42 g, 58.1 mmol) and *o*-toluidine (7.10 g, 66 mmol) was obtained 7.60 g (52%) of **3a**, mp 178–179 °C (lit.[15] mp 178–179 °C). ^{1}H NMR (270 MHz, $CDCl_3$) δ 2.12 (s, 3H), 2.25 (s, 3H), 7.10 (d, 1H, J = 7.0 Hz), 7.37 (m, 4H), 8.59 (dd, 1H, J = 1.9, 7.9 Hz), 8.98 (dd, 1H, J = 1.9, 4.6 Hz).

4.5. 2-Methyl-3-(2-chlorophenyl)pyrido[2,3-*d*]-4(3*H*)-pyrimidinone (3b)

From (**7**) (8.79 g, 54.2 mmol) and 2-chloroaniline (7.02 g, 55 mmol) was obtained 10.8 g (73%) of (**3b**), mp 192–193 °C (lit.[15] mp 194–195 °C). ^{1}H NMR (270 MHz, $CDCl_3$) δ 2.31 (s, 3H), 7.42 (m, 3H), 7.52 (m, 1H), 7.79 (dd, 1H, J = 1.4, 6.3 Hz), 8.60 (dd, 1H, J = 2.0, 7.6 Hz), 9.00 (dd, 1H, J = 2.0, 4.6 Hz).

4.6. General procedure for the acylation of 2-methyl-3-arylpyrido[2,3-*d*]-4(3*H*)-pyrimidinones

A 250 mL three-neck round-bottom flask was equipped with a stopper, a pressure-equalizing funnel and a condenser connected to a water-filled gas buret through a

drying tube filled with Drierite. The flask was charged with NaH (1.25 g of a 60% mineral oil dispersion, 31.3 mmol). The mineral oil was removed by washing several times with hexane followed by decantation and the NaH was immediately covered with dry THF (75 mL). Ethyl isonicotinate (1.00 g, 6.60 mmol) was then added, the stirred mixture was heated to reflux, the gas buret was adjusted to zero volume, and a solution of 2-methyl-3-(2-methylphenyl)pyrido[2,3-*d*]-4(3*H*)-pyrimidinone (**3a**) (1.51 g, 6.00 mmol) in THF (75 mL) was added dropwise over a period of 20 min. Hydrogen evolution commenced immediately and continued throughout the addition period. The resulting red-brown reaction mixture was heated at reflux until the theoretical amount of hydrogen had evolved, ca. 4 h, and then cooled to 0 °C in an ice bath and acetic acid (2.0 mL) was then cautiously added dropwise. The solvent was removed under reduced pressure and the solid residue was partitioned between CH_2Cl_2 and water. The layers were separated, the organic layer was washed with saturated $NaHCO_3$ solution, dried ($MgSO_4$) and concentrated to afford a brown semisolid, which was chromatographed on silica gel. Elution with CH_2Cl_2 gave a small amount of the starting ester. The product was then eluted with CH_2Cl_2:AcOEt 1:1 as a yellow solid, mp 222–224 °C, 1.61 g (75%). Recrystallization from AcOEt afforded an analytical sample of 2-[2-oxo-2-(4-pyridyl)ethyl]-3-(2-methylphenyl)pyrido [2,3-*d*]-4(3*H*)-pyrimidinone (**4a**), mp 225–226 °C. ^{1}H NMR (360 MHz, $CDCl_3$) δ 2.19 (s, 3H), 5.10 (s, 1H), 7.26 (m, 2H), 7.45 (m, 5H), 8.42 (dd, 1H, $J = 2.0$, 7.8 Hz), 8.61 (dd, 2H, $J = 1.6$, 4.5 Hz), 8.71 (dd, 1H, $J = 2.0$, 4.8 Hz), 14.97 (s, 1H). ^{13}C NMR (360 MHz, $CDCl_3$) δ 17.3, 81.3, 112.0, 120.3, 120.6, 128.1, 128.3, 130.4, 131.9, 133.9, 136.0, 137.2, 146.0, 150.2, 150.6, 154.8, 155.9, 159.6, 185.1. Anal. Calcd for $C_{21}H_{16}N_4O_2$: C, 70.78; H, 4.53; N, 15.72. Found: C, 70.64; H, 4.61; N, 15.74.

4.7. 2-[2-Oxo-2-(4-pyridyl)ethyl]-3-(2-chlorophenyl)pyrido[2,3-*d*]-4(3*H*)-pyrimidinone (4b)

From (**3b**) (1.83 g, 6.74 mmol), NaH (1.22 g, 51 mmol), and ethyl isonicotinate (1.24 g, 8.00 mmol) was obtained 1.17 g (46%) of **4b**, mp 216–217 °C. ^{1}H NMR (360 MHz, $CDCl_3$) δ 5.06 (s, 1H), 7.24 (m, 2H), 7.40 (m, 2H), 7.55 (m, 1H), 7.63 (m, 1H), 7.84 (dd, 1H, $J = 1.2$, 5.4 Hz), 8.42 (dd, 1H, $J = 1.8$, 4.6 Hz), 8.62 (dd, 2H, $J = 1.8$, 4.4 Hz), 8.72 (dd, 1H, $J = 1.9$, 7.6 Hz), 14.87 (s, 1H). Anal. Calcd for $C_{20}H_{13}N_4O_2Cl$: C, 63.75; H, 3.48; N, 14.87. Found: C, 63.90; H, 3.53; N, 14.66.

4.8. 2-[2-Oxo-2-(4-pyridyl)ethyl]-3-(2-bromophenyl)pyrido[2,3-*d*]-4(3*H*)-pyrimidinone (4c)

From (**3c**) (2.43 g, 7.68 mmol), NaH (1.51 g, 62.9 mmol), and ethyl isonicotinate (1.40 g, 9.26 mmol) was obtained 1.02 g (32%) of **4c**, mp 218–219 °C. ^{1}H NMR (360 MHz, $CDCl_3$) δ 5.05 (s, 1H), 7.29 (m, 2H), 7.43 (m, 2H), 7.52 (m, 1H), 7.60 (m, 1H), 7.87 (dd, 1H, $J = 1.1$, 5.7 Hz), 8.43 (dd, 1H, $J = 1.8$, 4.5 Hz), 8.63 (dd, 2H, $J = 1.6$, 4.4 Hz), 8.73 (dd, 1H, $J = 1.8$, 7.6 Hz), 14.19 (s, 1H). Anal. Calcd for $C_{20}H_{13}N_4O_2Br$: C, 57.03; H, 3.11; N,13.30. Found: C, 57.23; H, 3.17; N, 13.35.

4.9. 2-[2-Oxo-2-(4-pyridyl)ethyl]-3-phenylpyrido[2,3-*d*]-4(3*H*)-pyrimidinone (4d)

From **3d** (2.30 g, 9.69 mmol), NaH (1.25 g, 52.0 mmol), and ethyl isonicotinate (1.66 g, 11.0 mmol) was obtained 1.83 g (55%) of **4d**, mp 204–205 °C. ^{1}H NMR (360 MHz, $CDCl_3$) δ 5.17 (s, 1H), 7.45 (m, 8H), 8.43 (dd, 1H, $J = 2.0$, 4.6 Hz), 8.62 (dd, 2H, $J = 1.5$, 4.3 Hz), 8.73 (dd, 1H, $J = 2.0$, 7.6 Hz), 15.00 (s, 1H). Anal. Calcd for $C_{20}H_{14}N_4O_2$: C, 70.17; H, 4.12; N, 16.37. Found: C, 70.09; H, 4.10; N, 16.24.

4.10. 2-(2-Oxo-2-phenylethyl)-3-(2-methylphenyl)pyrido[2,3-*d*]-4(3*H*)-pyrimidinone (4e)

From (**3a**) (3.20 g, 12.7 mmol), NaH (2.30 g, 95.8 mmol), and methyl benzoate (2.83 g, 20.8 mmol) was obtained 2.72 g (60%) of **4e**, mp 216.5–217 °C. ^{1}H NMR (360 MHz, $CDCl_3$) δ 2.18 (s, 3H), 5.09 (s, 1H), 7.17 (m, 2H), 7.29 (m, 2H), 7.39 (m, 4H), 7.55 (m, 2H), 8.41 (dd, 1H, 1.6, 7.6 Hz), 8.69 (dd, 1H, 1.6, 5.0 Hz), 14.92 (s, 1H). Anal. Calcd for $C_{22}H_{17}N_3O_2$: C, 74.35; H, 4.82; N, 11.82. Found: C, 74.43; H, 4.89; N, 11.87.

4.11. 2-[2-(4-Pyridyl)ethenyl]-3-(2-methylphenyl)pyrido[2,3-*d*]-4(3*H*)-pyrimidinone (5b)

This compound was prepared in 97% yield according to a literature procedure,[15] mp 205–206 °C. ^{1}H NMR (360 MHz, $CDCl_3$) δ 2.14 (s, 3H), 6.32 (s, 1H), 6.38 (s, 1H), 7.36 (m, 7H), 8.33 (dd, 1H, $J = 1.8$, 7.4 Hz), 8.61 (dd, 2H, $J = 1.6$, 4.8 Hz), 9.02 (dd, 1H, $J = 1.8$, 4.6 Hz). ^{13}C NMR (360 MHz, $CDCl_3$) δ 17.6, 116.4, 122.4, 122.5, 127.9, 128.4, 130.3, 131.8, 135.1, 135.9, 136.8, 139.9, 142.1, 150.3, 154.0, 156.7, 157.7, 161.9. Anal. Calcd for $C_{21}H_{16}N_4O$: C, 74.10; H, 4.74; N, 16.46. Found: C, 74.40; H, 4.79; N, 16.29.

4.12. Pharmacology

Anticonvulsant evaluation of candidate compounds **3**–**5** was conducted by the Epilepsy Branch of NINDS using standard protocol (see Ref. 4).

4.13. In vitro biological assays

Methods for chloride flux assay of $GABA_A$ receptor function were similar to those of Bloomquist and Soderlund,[11] except that 15 s incubations with agonist were used. For presynaptic [^{3}H]serotonin release studies, we essentially followed the methods of Kirby et al.,[13] except that the mice used in the present study were albino male ICR strain. Concentration–response curves were replicated at least 3 times and were analyzed by nonlinear regression to a four parameter logistic equation to determine EC_{50} and maximal uptake parameters using Prism (GraphPad Software, San Diego, CA). Single concentration experiments were also replicated 3 times and analyzed by T-test or ANOVA using InStat (GraphPad Software, San Diego, CA).

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