Highly Selective κ Opioid Analgesics. Synthesis and Structure–Activity Relationships of Novel *N*-[(2-Aminocyclohexyl)aryl]acetamide and *N*-[(2-Aminocyclohexyl)aryloxy]acetamide Derivatives

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This paper describes the synthesis, structure–activity relationships (SAR) of μ and κ opioid binding affinities, and analgesic properties of a series of novel highly selective κ opioid *N*-[(2-aminocyclohexyl)aryl]acetamide and *N*-[(2-aminocyclohexyl)aryloxy]acetamide derivatives. Ten compounds, **14**, **15**, **31–37**, and **39** (Tables I and II), show a marked κ selectivity of greater than 100:1 over μ binding, with high affinity for the κ opioid receptor ($\sim 10^{-8}$–10^{-9} M). Compound **39**, (*S,S-trans*)-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]-4-benzo[*b*]furanacetamide hydrobromide, has the highest μ/κ selectivity, 780:1 (κK_i = 4.2 nM), reported to date. Four of these compounds, **14**, **15**, and their *S,S*-trans enantiomers, **37** and **39**, respectively, produce effective analgesia by *oral* administration, as assayed by a rat-paw pressure test (RPP) (MPE_{50} = 24, 26, 8.3, and 12 mg/kg, respectively). The *R,R*-trans isomer, **38**, was inactive in binding and RPP. The analgesic effect was reversed by administration of naloxone, confirming these effects are opioid in character. Optimal activity is produced when the basic nitrogen atom is in a pyrrolidine ring, the aryl group is a 10-π-electron-rich aromatic system, such as 4-benzo[*b*]thiophene, 4-benzo[*b*]furan, or 4-chlorophenoxy, and overall lipophilicity lies within the range log *P* = 3.5–5.0.

The development of a potent analgesic agent with minimal abuse liability, for the treatment of pain arising from all etiologies, has been a major aim of drug research for many decades. These endeavors have focused mainly on chemical modification of the prototype opioid agonist, morphine. However, the structure–activity relationships (SAR) observed among derivatives of morphine[1,2] may now need further refinement since the discovery of multiple opioid receptors. Evidence for the existence of μ, κ, and δ subtypes of opioid receptors has received much support from binding and pharmacological studies.[3–8] Activation of μ receptors appears to be associated with classical morphine-like properties, such as centrally mediated analgesia, constipation, respiratory depression, and physical dependence.[1,9] Activation of κ receptors appears to be associated with centrally mediated analgesia, sedation, and diuresis.[9]

Examples of diverse chemical classes of compounds that have been described as selective κ ligands include the endogenous peptides, the dynorphins **1**,[7,10] the benzodiazepine derivative tifluadom (**2**),[11] the 6,7-benzomorphan derivative ethylketocyclazocine (**3**),[5,8,12] and the newer cyclohexylbenzeneacetamide derivatives **4**, such as U-50488 (**4a**),[13–16] U-69593 (**4b**),[17] and U-62066 (spiradoline, **4c**)[18] (Figure 1). The cyclohexylbenzeneacetamides **4** are of interest because (i) they are structurally unrelated to morphine, (ii) they are the most selective κ ligands hitherto described, (iii) they are well absorbed and orally effective analgesics in animal models, and (iv) they appear not to be self-administered or induce opiate-like physical dependence.[13–16] These characteristics make this new chemical class worthy of further investigation, as these compounds appear to have potential as therapeutically useful opioid analgesics with minimal abuse liability in humans.

The objectives of this study have been to optimize the affinity and μ/κ selectivity of compounds with the general formula **7** (Figure 1) for the κ opioid receptor. This paper describes the synthesis, SAR of μ and κ opioid binding affinities, and analgesic properties of a series of novel *N*-[(2-aminocyclohexyl)aryl]acetamides[19] and *N*-[(2-aminocyclohexyl)aryloxy]acetamides.[20] A number of chemical variations have been made to the amide side chain, the length of bridging chain to the aryl and aryloxy substituents (Ar), and the amino side chain (R^1, R^2) (Table I). An SAR for these series has been established from the biological data in Table II. Ten compounds with μ/κ selectivity of greater than 100 have been identified.

Synthesis

Compounds in Table I were prepared by methods A–D as outlined in Scheme I. 7-Methyl-7-azabicyclo[4.1.0]-heptane (**5**) was treated with the primary or secondary amines to give the racemic *trans*-1,2-diamines **6**. These

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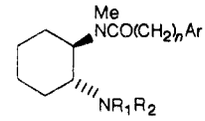
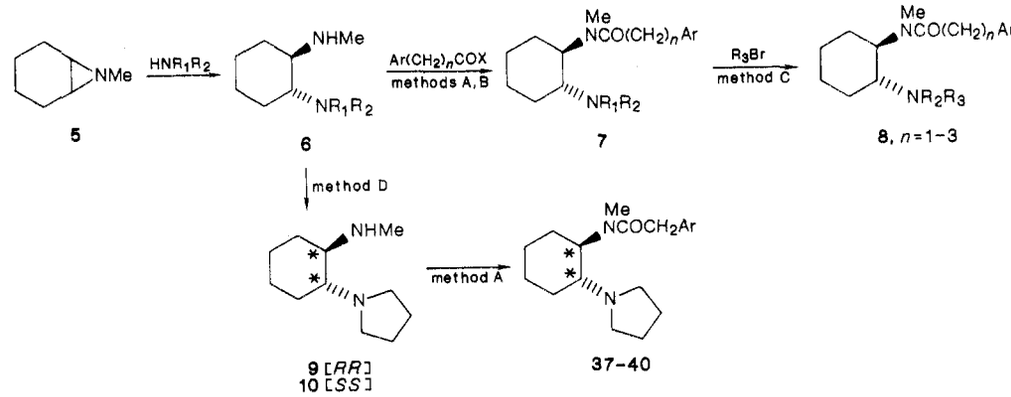
Table I. Physical Properties of Compounds 11–40



no.	formula	Ar	R_1R_2	n	mp, °C	coupling[a] method	analysis	crystallization solvent
11	$C_{17}H_{25}N_2OS\cdot HCl\cdot 0.1H_2O$	2-thiopheneyl	$(CH_2)_4$	1	165–167	A	C, H, N, Cl	CH_2Cl_2
12	$C_{19}H_{28}N_2O_2\cdot HCl\cdot 0.1H_2O$	phenoxy	$(CH_2)_4$	1	193–196	A	C, H, N, Cl	Et_2O–CH_2Cl_2
13	$C_{21}H_{29}N_3O\cdot 0.2H_2O$	3-indolyl	$(CH_2)_4$	1	188.5–190	A	C, H, N	EtOAc–MeOH
14	$C_{21}H_{28}N_2O_2HCl$	4-benzo[*b*]furanyl	$(CH_2)_4$	1	234.5–242.5	A	C, H, N	Et_2O–CH_2Cl_2
15	$C_{21}H_{28}N_2OS\cdot HCl$	4-benzo[*b*]thiopheneyl	$(CH_2)_4$	1	252–254	A	C, H, N, Cl	EtOAc–CH_2Cl_2
16	$C_{19}H_{27}ClN_2O_2\cdot HCl\cdot 0.8H_2O$	4-Cl-phenoxy	$(CH_2)_4$	1	179–180.5	A	C, H, N, Cl	Et_2O–CH_2Cl_2
17	$C_{19}H_{26}Cl_2N_2O_2\cdot HCl$	3,4-Cl_2-phenoxy	$(CH_2)_4$	1	227–229	A	C, H, N, Cl	CH_2Cl_2
18	$C_{19}H_{26}Cl_2N_2O_2\cdot HCl\cdot 0.5H_2O$	2,6-Cl_2-phenoxy	$(CH_2)_4$	1	173–175	A	C, H, N, Cl	Et_2O–CH_2Cl_2
19	$C_{20}H_{26}F_3N_3O_4\cdot HCl\cdot 0.3H_2O$	3-CF_3-4-NO_2-phenoxy	$(CH_2)_4$	1	190–192	A	C, H, N, Cl	CH_2Cl_2–Et_2O
20	$C_{19}H_{25}Cl_3N_2O_2\cdot HCl\cdot H_2O$	3,4,5-Cl_3-phenoxy	$(CH_2)_4$	1	188–190	A	C, H, N	CH_2Cl_2–Et_2O
21	$C_{19}H_{25}Cl_3N_2O_2\cdot HCl$	2,4,6-Cl_3-phenoxy	$(CH_2)_4$	1	159–161	A	C, H, N, Cl	CH_2Cl_2–Et_2O
22	$C_{22}H_{31}N_3O\cdot 0.1H_2O$	3-indolyl	$(CH_2)_4$	2	145.5–145.6	A	C, H, N	hexane–EtOAc
23	$C_{23}H_{33}N_3O\cdot H_2SO_4$	3-indolyl	$(CH_2)_4$	3	106–112	B	C, H, N[b]	EtOH–Et_2O
24	$C_{22}H_{30}N_2OS\cdot HCl$	4-benzo[*b*]thiopheneyl	$(CH_2)_5$	1	221–231	B	C, H, N, Cl	CH_2Cl_2–Et_2O
25	$C_{23}H_{32}N_2OS\cdot HCl$	4-benzo[*b*]thiopheneyl	$(CH_2)_6$	1	224–236	A	C, H, N, Cl	CH_2Cl_2–Et_2O
26	$C_{21}H_{30}N_2OS\cdot HCl$	4-benzo[*b*]thiopheneyl	Et, Et	1	122–126	A	C, H, N, Cl	CH_2Cl_2–Et_2O
27	$C_{24}H_{30}N_2OS_2\cdot HCl$	4-benzo[*b*]thiopheneyl	Me, [2-(thiophene-2-yl)ethyl]	1	173–178	A	C, H, N	CH_2Cl_2–Et_2O
28	$C_{26}H_{32}N_2OS\cdot HCl$	4-benzo[*b*]thiopheneyl	Me, phenethyl	1	189–193	A	C, H, N, S[c]	CH_2Cl,–Et_2O
29	$C_{22}H_{30}N_2OS\cdot HCl$	4-benzo[*b*]thiopheneyl	Me, CH_2-c-C_3H_5	1	229–234	C	C, H, N, Cl	CH_2Cl_2–Et_2O
30	$C_{21}H_{28}N_2OS\cdot HCl$	4-benzo[*b*]thiopheneyl	Me, allyl	1	209–211.5	C	C, H, N	CH_2Cl_2–Et_2O
31	$C_{21}H_{30}Cl_2N_2O_2\cdot HCl\cdot 0.9H_2O$	3,5-Cl_2-phenoxy	$(CH_2)_6$	1	180–185	A	C, H, N	CH_2Cl_2–Et_2O
32	$C_{22}H_{31}Cl_3N_2O_2\cdot HCl\cdot 0.7H_2O$	3,4,5-Cl_3-phenoxy	$(CH_2)_7$	1	194–200	A	C, H, N, Cl	CH_2Cl_2–Et_2O
33	$C_{22}H_{30}Cl_4N_2O_2\cdot HCl\cdot 0.8H_2O$	2,3,4,5-Cl_4-phenoxy	$(CH_2)_7$	1	179–183	A	C, H, N	CH_2Cl_2–Et_2O
34	$C_{21}H_{28}Cl_4N_2O_2\cdot HCl$	2,3,4,5-Cl_4-phenoxy	$(CH_2)_6$	1	185–188	A	C, H, N	CH_2Cl_2–Et_2O
35	$C_{23}H_{32}N_2O_2\cdot HCl$	4-benzo[*b*]furanyl	$(CH_2)_6$	1	224–234	A	C, H, N	CH_2Cl_2–Et_2O
36	$C_{23}H_{35}ClN_2O_2\cdot HCl$	4-Cl-3,5-Me_2-phenoxy	$(CH_2)_6$	1	165–169	A	C, H, N, Cl	MeOH
37	$C_{21}H_{28}N_2OS\cdot HCl\cdot 0.6H_2O$ (–)	4-benzo[*b*]thiopheneyl	$(CH_2)_4$	1	136–146	D, A	C, H, N	MeOH–Me_2CHOH
38	$C_{21}H_{28}N_2OS\cdot HCl\cdot 0.6H_2O$ (+)	4-benzo[*b*]thiopheneyl	$(CH_2)_4$	1	137–142	D, A	C, H, N	MeOH–Me_2CHOH
39	$C_{21}H_{28}N_2O_2\cdot HBr\cdot 0.8H_2O$ (–)	4-benzo[*b*]furanyl	$(CH_2)_4$	1	115–125	D, A	C, H, N	CH_2Cl_2–Et_2O
40	$C_{21}H_{28}N_2O_2\cdot HCl\cdot 0.7H_2O$ (+)	4-benzo[*b*]furanyl	$(CH_2)_4$	1	128–138	D, A	C, H, N	Et_2O–CH_2Cl_2

[a] See the Experimental Section. [b] $C_{23}H_{32}N_3O\cdot H_2SO_4$ requires: C, 59.33; H, 7.58; N, 9.02. Found: C, 61.46; H, 7.16; N, 9.06. [c] $C_{26}H_{32}N_2OS\cdot HCl$ requires: C, 68.32; H, 7.28; N, 6.13; S, 7.01. Found: C, 65.56; H, 7.16; N, 6.33; S, 6.71.

Scheme I[a]



[a] Method A, R_1, $R_2 \neq H$, X = Cl; method B, R_1, $R_2 \neq H$, X = imidazole; method C, R_1 = H, R_2 = CH_3, alkylation by R_3Br; method D, R_1, R_2 = c-$(CH_2)_4$, (i) 2,3-di-*p*-toluoyl-D-tartaric acid, (ii) KOH.

were coupled with the appropriate carboxylic acid via the acid chloride (method A) or the acylimidazole (method B) to give the *N*-acyl derivatives **7**. Subsequent N-alkylations were carried out by treatment of the secondary amines with an alkyl halide (method C, R_1 = H) to give **8**. The diamine obtained by opening the aziridine with pyrrolidine (**6**, R_1, R_2 = c-C_4H_8) was resolved by repeated fractional crystallization of the 2,3-di-*p*-toluoyl-D-tartaric acid salt (method D), and the two enantiomeric diamines **9** and **10** were used to prepare the optically active compounds **37–40**.

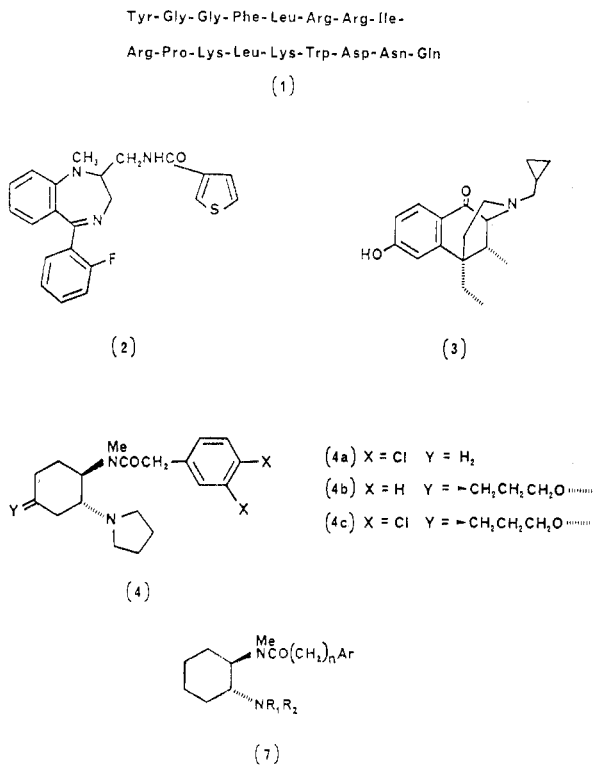


Figure 1. Structures of compounds **1–3**, **4a–c**, and **7**.

Results and Discussion

1. SAR of the Amide Side Chain. The starting point for this study was consideration of the properties of the 3,4-dichlorophenyl moiety in the benzene acetamide derivative U-50488 (**4a**) (Figure 1). The physicochemical parameters required for κ binding of this compound are not immediately apparent, as electronic, lipophilic, and steric components may be important.

Firstly, in order to test the electronic requirement, examples of electron-rich and electron-deficient aromatic rings were prepared. Binding data on the electron-rich thiophene derivative **11** (Table I) were encouraging because significant affinity (K_i = 720 nM) and selectivity (μ/κ ratio = 25) for the κ opioid receptor were observed. Binding data on electron-rich phenoxy (K_i = 360 nM) derivatives **12** further support the preference for an electron-rich aryl group.

Secondly, the lipophilic requirement was explored. The thiophene derivative **11** described above has log *P* (calcd) = 3.1. (See the Experimental Section, and ref 27). log *P* calculated in the same way for **4a** was found to be 4.70. In order to explore the variation of activity with increasing lipophilicity, a series of analogues with the aryl substituents 3-indolyl (**13**) (log *P* (calcd) = 3.4), 4-benzo[*b*]furanyl (**14**) (log *P* (calcd) = 3.9), and 4-benzo[*b*]thiopheneyl (**15**) (log *P* (calcd) = 4.4) were prepared. Examination of Dreiding and CPK molecular models revealed that these benzo-fused heterocyclic rings introduced little steric difference compared with the 3,4-dichlorophenyl moiety present in **4a**. Binding data show that the 4-benzo[*b*]thiophene and benzo[*b*]furan derivatives **15** and **14** have both excellent κ affinities (K_i = 3.7 and 12 nM, respectively) and selectivities (μ/κ ratio = 110 and 130, respectively). Moreover, good analgesia, as assayed by the rat-paw pressure (RPP) assay (MPE_{50} = 26 and 24 mg/kg po, respectively), was observed on oral administration of these compounds.

These physicochemical parameters were further explored in the phenoxy series, where a range of the precursor substituted phenoxyacetic acids are readily available. In a "Topliss decision-tree"[21] study, the 4-chlorophenoxy derivative **16** was found to have modest increased affinity relative to the unsubstituted derivative **12** for the κ receptor (K_i = 300 and 360 nM, respectively). The next analogue suggested in the "Topliss tree", the 3,4-dichloro analogue **17**, had a 10-fold increase in affinity (K_i = 35 nM) over the unsubstituted compound. The 2,6-dichloro analogue **18** (K_i = 2100 nM) is considerably less active than compound **17**, indicating that κ receptor recognition is sensitive to the ortho substitution in this aromatic moiety. The 4-nitro-3-(trifluoromethyl) analogue **19** (K_i = 150 nM) was also found to be less active than **17**. This suggests that the $+\pi$, rather than the $+\sigma$ effect of the chlorine substituents is primarily responsible for increased potency.[21] In order to exploit this observation, the effect of introducing a third chlorine substituent was investigated. The 3,4,5-trichloro derivative **20** showed a further increase in κ affinity (K_i = 12 nM), but again, when two chlorine substituents were placed ortho to the phenoxy oxygen atom in the 2,4,6-trichloro derivative **21**, potency was decreased dramatically (K_i = 10 000 nM). Finally, the effect of varying the chain length between the aromatic group and the amide function was probed by testing the series **13**, **22**, and **23** (Table I, *n* = 1–3). The binding data (Table II)

Table II. μ and κ Opioid Binding Affinity and Rat-Paw Pressure Assay

	opioid receptor binding affinity: K_i,[a] nM			rat-paw pressure assay: MPE_{50}, mg/kg		lipophilicity: log *P* (calcd)
no.	κ	μ	μ/κ	po	sc[b]	
11	720	18000	25	[c]	[c]	3.1
12	360	>1000		[c]	[c]	2.9
13	74	2900	39	[c]	>300	3.4
14	12	1600	130	24	[c]	3.9
15	3.7	410	110	26	10	4.4
16	300	2000	6.7	59	210	3.6
17	35	620	18	[c]	240	4.3
18	2100	4000	1.9	110	[c]	4.3
19	150	>1000		>100	[c]	3.5
20	12	140	12	[c]	[c]	5.0
21	>10000	>10000		[c]	[c]	5.0
22	>1000	>1000		[c]	[c]	3.9
23	>1000			[c]	[c]	4.4
24	250	2400	9.6	[c]	[c]	4.9
25	40	810	20	120	>100	5.4
26	1800	350	0.19	[c]	[c]	4.4
27	82	81	0.99	>300	[c]	5.7
28	30	26	0.87	[c]	>100	6.0
29	200	190	0.95	[c]	[c]	4.9
30	52	210	4.0	[c]	[c]	4.3
31	72	7900	110	>100	[c]	5.3
32	3.1	880	280	>100	[c]	6.5
33	5.6	3600	640	>300	[c]	7.2
34	6.4	720	110	>300	[c]	6.7
35	29	3500	120	>100	[c]	4.9
36	4.0	460	120	[c]	[c]	6.3
37	9.6	1000	100	8.3	[c]	4.4
38	1500	880	0.59	>100	[c]	4.3
39	4.2	3300	780	12	[c]	3.9
40	1500	1400	0.93	[c]	[c]	3.9
4a	10	880	88	43	[c]	4.7
4b	7.3	52	7.1	[c]	[c]	
4c	17	6400	380	7.8	[c]	
3	0.57	1.7	3.0	70	0.30	
morphine	86	1.8	0.02	13	1.4	

[a] Each K_i value represents the mean from concentration–response curves performed in triplicate. [b] MPE_{50} values represent the dose required to produce 50% of the maximum possible analgesic effect. They are derived from a single experiment with six animals at each of five dose levels. [c] Not tested.

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show that the single methylene linkage in compound **13** is optimal.

2. SAR of the Amine Side Chain. The SAR of the amine side chain was independently explored while the 4-benzo[*b*]thiophene methylene moiety was maintained on the amide side chain.

Increasing the size of the ring that contains the amino nitrogen from pyrrolidine to hexahydro-1*H*-azepine (five- to seven-membered rings) (**15**, **24**, **25**) causes a considerable variation in affinity and selectivity (K_i = 4, 250, and 40 nM; μ/κ ratio = 110, 10, and 20, respectively) in the order pyrrolidine > hexahydro-1*H*-azepine > piperidine. The dramatic difference in κ affinity and selectivity for the pyrrolidine compound **15** [K_i (κ) = 4 nM, (μ) = 410 nM] and its "ring-opened congener", the diethyl compound **26** (K_i (κ) = 1800 nM, (μ) = 350 nM), also shows that the κ receptor, unlike the μ receptor, is highly sensitive to steric effects in this part of the molecule.

In order to compare further the SAR requirements of the κ and μ receptors, compounds **27**–**30** were prepared. Compounds **27** and **28** contain the *N*-[2-(thiopheneyl)-ethyl] and *N*-(2-phenylethyl) moieties, which are present as substituents on the amine moiety in the μ selective analgesics sufentanil and fentanil.[22] Compounds **29** and **30** contain the *N*-(cyclopropylmethyl) and *N*-2-propenyl groups, which are present as substituents on the amine moiety in the opiate antagonists naltrexone and naloxone, respectively.[1] In this series of (aminocyclohexyl)arylacetamides, these amino substituents do not improve κ affinity. Indeed, compared with the pyrrolidinyl analogue **15**, they actually enhance the μ receptor affinity (Table II). These data indicate that the κ receptor requires a different set of SAR on the basic nitrogen than the μ receptor.

Among the polysubstituted phenoxy aryl series, compounds **31**–**36** all show *greater* than 100-fold μ/κ selectivity. It is interesting to note that the chlorinated (aryloxy)-acetamides **31**–**33** and **36** exhibit higher κ selectivity when the amino moiety constitutes a seven- or eight-membered ring, instead of a five-membered ring. In this respect, the SAR of the (aryloxy)acetamide series differs from that described above for the arylacetamide series.

3. Enantiomers of Compounds 14 and 15. The analgesic activity of morphine and other chiral μ opiates resides mainly in one enantiomer.[1] Compounds **15** and **14** were therefore resolved into their two enantiomers (**37**, **38** and **39**, **40**, respectively) by repeated fractional crystallization of the diamine **6** (R_1, R_2 = c-C_4H_8) 2,3-di-*p*-toluoyl-D-tartrate salts, to give **9** and **10**, which were acylated and shown to be >98% enantiomerically pure by the chiral solvating agent NMR method (see the Experimental Section). The κ binding affinity, μ/κ selectivity, and analgesic activity have been found to reside in the (−) enantiomers (**37** and **39** K_i (κ) = 9.6 and 4.2 nM, μ/κ ratio = 100:1 and 780:1, respectively, MPE_{50} = 8.3 and 12 mg/kg, po) with little activity in the corresponding (+) enantiomers (**38** and **40** K_i (κ) = 1500 and 1500 nM, respectively, MPE_{50} = >100 mg/kg, po for **38**).

The (−) enantiomer of the 4-benzo[*b*]furan analogue **39** has a μ/κ selectivity of 780 and is the most selective κ analgesic compound reported to date. The X-ray crystal structure of the active 4-benzo[*b*]thiophene enantiomer **37** (Figure 2) shows the absolute stereochemistry to be 1,2-[*S*,*S*], consistent with that assigned for spiradoline.[18] Furthermore, the X-ray (Figure 2) indicates that the pyrrolidine ring in this active enantiomer, **37**, is approximately orthogonal to the plane of the cyclohexane ring. Examination of Dreiding and CPK molecular models indicates a severe steric interaction between the adjacent methylene hydrogen atoms of the cyclohexane ring and the NCH_2 hydrogen atoms of the pyrrolidine ring, which is relieved when the rings are approximately orthogonal.

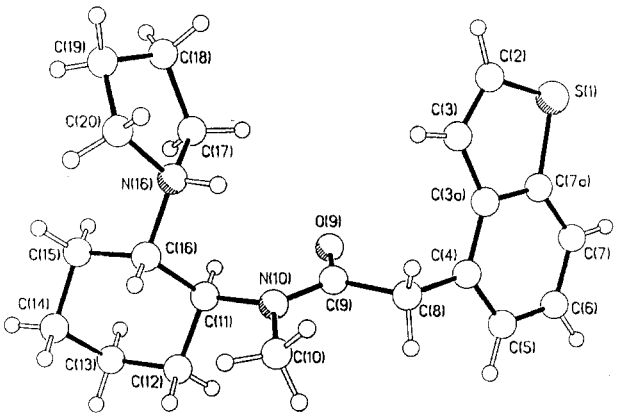

Figure 2. X-ray crystal structure of compound **37**.

Oral Activity

Compounds **14**, **15**, and **4a** have high κ binding affinity and show analgesia on oral administration to rats. However, compounds **31**–**34** also with high κ affinity and selectivity are inactive on oral administration (Table II). Compounds **14**, **15**, and **4a** have log *P* (calcd) = 3.9–4.7, whereas the orally inactive compounds **31**–**34** have a higher log *P* (calcd) = 5.3–7.2. Compounds with log *P* (calcd) < 3.5 have poor κ affinity (e.g., **11**, **12**, and **19**) and, for compound **19**, no oral analgesia.

From these limited data, it appears that in order to achieve *oral* efficacy, these (diaminocyclohexyl)arylacetamides[19,20] should have overall lipophilicity in the range log *P* (calcd) = 3.5–5.0.

The analgesic effects of **15** were shown to be opioid in origin by their complete reversal on administration of the opioid antagonist naloxone.

Conclusion

This study confirms that the *N*-(2-aminocyclohexyl)-acetamides (Figure 1) are the most selective chemical class of κ opioid ligands yet described. Furthermore, the 10 novel derivatives, **14**, **15**, **31**–**37**, and **39**, have a μ/κ selectivity greater than 100 and high affinity for the κ receptor. Compound **39** has the highest selectivity (780:1) reported to date. Four of these compounds, **15**, **14**, and their corresponding (−) enantiomers, **37** and **39**, respectively, produce analgesic effects by oral administration. This effect was shown to be reversed by administration of naloxone and hence opioid in origin.

It has been found that these ligands exhibit analgesia when log *P* (calcd) lies in the range 3.5–5.0. Compounds with high κ selectivity (e.g., **32**, **33**) but high log *P* (calcd) (6.5 and 7.2, respectively) are inactive when administered orally, probably because they are not well absorbed.

It appears that for optimal selectivity and oral efficacy in this series, the following criteria are required: (i) the overall lipophilicity should be in the range measured by log *P* (calcd) ~ 3.5–5.0; (ii) the aromatic moiety should possess an electron-rich 10-π system; (iii) the amino nitrogen should be incorporated in a ring, preferably pyrrolidine; and (iv) the arylamide or aryloxyamide group linking chain has one methylene group.

The development of more selective opioid ligands may provide further tools to test these criteria and clarify the

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SAR requirements of the κ and μ subtypes of opioid receptors. Such studies could lead ultimately to a new generation of potent analgesics with minimal abuse liability in humans.

Experimental Section

Biological Assays. 1. μ and κ Receptor Binding Assay. Measurement of the κ opioid receptor site binding activity of compounds was made by the method of Gillan et al.[23] Guinea pig brain homogenates were prepared fresh each day. The binding of tritiated etorphine to brain homogenates was measured in the presence of unlabeled competitor compounds with 200 nM [D-Ala^2,D-Leu^5]enkephalin (acronym DADLE) and 200 nM [D-Ala^2,*N*-MePhe^4,Gly-ol^5]enkephalin (acronym DAGO) added to saturate the δ and μ opioid receptors, respectively. The reaction was terminated by rapid filtration, and the radioactivity bound to the filters was counted by liquid scintillation spectrophotometry. Measurement of the μ opioid receptor site binding activity of compounds was made by the following method. Guinea pig brain homogenates were prepared fresh each day via the method of Gillan et al. cited above. Homogenates were incubated for 150 min at 0 °C with tritiated DAGO to measure μ receptor site binding activity. Nonspecific binding was determined in the presence of 10^{-6} M DAGO and 10^{-6} M DADLE.

Reactions were terminated by rapid filtration, and the radioactivity bound to the filters was counted by liquid scintillation spectrophotometry. The inhibition of binding of tritiated etorphine, DAGO, and DADLE by cold ligands was determined from the regression of log percentage inhibition of specific binding or log concentration of cold ligand. The inhibition constant (K_i) was calculated from the equation:

$$K_i = IC_{50}/(1 + [L]/K_D)$$

where [L] is the concentration of the labeled ligand and K_D, its equilibrium dissociation constant.

2. Analgesia Assay. The rat-paw pressure test (RPP) was performed by the method of Tyers.[24]

Chemistry. (a) General Procedures. Melting points were determined with a Reichart Thermovar hot-stage apparatus and are uncorrected. Proton NMR spectra were recorded on a Bruker AM 300 or JEOL PMX-60SI spectrometer; chemical shifts were recorded in parts per million downfield from tetramethylsilane. IR spectra were recorded with a Perkin-Elmer 1420 or 1750 spectrophotometer. Optical rotations were determined in dichloromethane solution with a Perkin-Elmer 241 polarimeter. Silica gel used for chromatography was Kieselgel-60 (230–400 mesh) (E. Merck A. G., Darmstadt, Germany).

The lipophilicity of **4a** was determined, by reversed-phase high-performance thin-layer chromatography (HPTLC), to be log *P* (obsd) = 4.7. This value was used as a reference for log *P* calculations by the method of Hansch and Leo[25] on further analogues.

(b) Synthesis of Intermediates. *trans*-1,2-Cyclohexanediamines **6** were prepared by the method of Szmuszkovicz.[14] The new phenoxyacetic acids **41–46** were prepared by the method of Dell[26] from the corresponding phenol. Compound **41** [4-nitro-3-(trifluoromethyl)phenoxy]acetic acid: 92%; mp 106–110 °C (toluene). Anal. ($C_9H_6F_3NO_5$) C, H, N. Compound **42** (3,4,5-trichlorophenoxy)acetic acid: 87%; mp 136–138 °C (toluene). Anal. ($C_8H_5Cl_3O_3$) C, H. Compound **43** (2,4,6-trichlorophenoxy)acetic acid: 82%; mp 170–171 °C (toluene). Anal. ($C_8H_5Cl_3O_3$) C, H. Compound **44** (3,5-dichlorophenoxy)acetic acid: 90%; mp 106–109 °C (toluene). Anal. ($C_8H_6Cl_2O_3$) C, H. Compound **45** (2,3,4,5-tetrachlorophenoxy)acetic acid: 84%; mp 174–177 °C (toluene). Anal. ($C_8H_4Cl_4O_3$) C, H. Compound **46** (4-chloro-3,5-dimethylphenoxy)acetic acid: 86%; mp 100–103 °C (EtOH–H_2O). Anal. ($C_{10}H_8O_3$) C, H. The other carboxylic acids and reagents obtained from commercial sources were used without further purification. 4-Benzo[*b*]furanacetic acid was prepared from 6,7-dihydro-4(5*H*)-benzofuranone[27] via methodology described by Kloetzel et al.[28] 4-Benzo[*b*]thiophene acetic acid was obtained from the Aldrich Chemical Co.

(c) Synthetic Procedures in Table I and Scheme I. Method A. *trans*-(±)-*N*-Methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]-2-(2,4,6-trichlorophenoxy)acetamide Monohydrochloride (21). A solution of (2,4,6-trichlorophenoxy)acetyl chloride (0.280 g, 1.02 mmol), prepared by the action of thionyl chloride on the acid, in dichloromethane (5 mL) was added dropwise to a stirred solution of *trans*-(±)-*N*-methyl-2-(1-pyrrolidinyl)cyclohexanamine (0.182 g, 1 mmol) in dichloromethane (5 mL) at 0 °C. After the mixture was stirred for 10 min, diethyl ether was added until no further precipitate resulted. The product was collected by filtration, washed with diethyl ether, and dried in vacuo at 60 °C to yield the amide hydrochloride (0.430 g, 93%): mp 150–161 °C; IR (Nujol mull) 2460 (br, N—H), 1635 (C=O), 1570, 1550 cm^{-1}; NMR (DMSO-d_6) δ 1.1–2.2 (12 H, m, CH_2's), 2.9 (3 H, s, NMe), 3.0–3.8 (5 H, m, C*H*N and 2 CH_2N), 4.55 (1 H, m, C*H*N), 4.8 (1 H, d, *J* = 13 Hz, one of the CH_2O), 4.9 (1 H, d, *J* = 13 Hz, one of CH_2O), 7.7 (2 H, s, aromatic), 10.25 (1 H, br d, NH). Anal. ($C_{19}H_{25}N_2O_2Cl_3$·HCl) C, H, N, Cl.

Method B. *trans*-*N*-Methyl-*N*-[2-(1-piperidinyl)cyclohexyl]benzo[*b*]thiophene-4-acetamide Monohydrochloride (24). Benzo[*b*]thiophene-4-acetic acid (0.50 g, 2.6 mmol) and carbonyldiimidazole (0.46 g, 2.8 mmol) were dissolved in tetrahydrofuran (5 mL) and heated to reflux for 0.5 h in an atmosphere of dry nitrogen. The resulting solution was cooled to room temperature, treated with a solution of *trans*-(±)-*N*-methyl-2-(1-piperidinyl)cyclohexanamine (0.46 g, 2.3 mmol) in tetrahydrofuran (2 mL) and heated to reflux for 5 min. The solvent was removed in vacuo to give an oil, which was poured into saturated aqueous sodium bicarbonate solution (30 mL) and extracted with dichloromethane (2 × 20 mL). Evaporation of the organic fractions left an oil, which after trituration with diethyl ether furnished *trans*-(±)-*N*-methyl-*N*-[2-(1-piperidinyl)cyclohexyl]benzo[*b*]-thiophene-4-acetamide as a white solid (0.61 g, 1.6 mmol, 72%), IR max (neat) 1635 cm^{-1}. A portion of this amide (0.37 g, 1.0 mmol) was dissolved in dichloromethane (3 mL) and diethyl ether (5 mL) and treated with a solution of hydrogen chloride in diethyl ether to give the hydrochloride salt (0.40 g, 0.98 mmol, 98%) as a white solid. An analytically pure sample was obtained by recrystallization (dichloromethane–diethyl ether), mp 221–231 °C.

Method C. *trans*-(±)-*N*-Methyl-*N*-[2-[methyl(2-propenyl)amino]cyclohexyl]benzo[*b*]thiophene-4-acetamide Monohydrochloride (30). A solution of *trans*-(±)-*N*-methyl-*N*-[2-(methylamino)cyclohexyl]benzo[*b*]thiophene-4-acetamide (0.358 g, 1.13 mmol) in ethanol (10 mL) and dichloromethane (1 mL) was treated with 3-bromo-1-propene (257 mg) and triethylamine (0.30 mL) and stirred in an oil bath at 50–60 °C for 24 h. The solvent was removed in vacuo to give a residue, which was poured into (10% w/v) aqueous potassium carbonate solution (50 mL). Extraction with dichloromethane (2 × 30 mL) gave an oil, which was purified by silica gel chromatography (100:1 ethyl acetate–triethylamine) to give *trans*-(±)-*N*-methyl-*N*-[2-[methyl(2-propenyl)amino]cyclohexyl]benzo[*b*]thiophene-4-acetamide (0.27 g, 67%), IR max (neat) 1637 cm^{-1}. This amide (0.27 g) was dissolved in diethyl ether (5 mL) and treated with a solution of hydrogen chloride in diethyl ether to give hydrochloride salt (0.29 g, 97%) as a white solid. An analytically pure sample was obtained by recrystallization (dichloromethane–diethyl ether), mp 209–211.5 °C.

Method D. Resolution of the Diamino Compounds 9 and 10. The enantiomers **9** and **10** of the diamine **6** (Scheme I; R_1, R_2 = c-C_4H_8) were separated by repeated fractional crystallization of the 2,3-di-*p*-toluoyl-D-tartaric acid salts as follows.

The racemic diamine (16 g, 88 mmol) and 2,3-di-*p*-toluoyl-D-tartaric acid (Aldrich Chemical Co.) (35.6 g, 88 mmol) were

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dissolved separately in methanol at 60 °C (400 + 400 mL) and mixed, the solution was cooled, and the resulting solid was collected by filtration. The solid (27.3 g) was put aside, and then the mother liquors were concentrated to 170 mL, cooled, and filtered to remove further solid, which was discarded. The resulting mother liquors were further concentrated to 120 mL, cooled, filtered (the solid discarded), and then evaporated to dryness. The resulting solid (23 g) was partitioned between dichloromethane and aqueous (20%) potassium hydroxide solution; the dichloromethane layer was evaporated to give crude parent amine, which was distilled in vacuo to yield the pure (+) enantiomer of the diamine as a colorless liquid: bp 116–119 °C (13 mm); 3.1 g (17 mmol, 19%); $[\alpha]^{20}_D$ (CH_2Cl_2) +91.5°.

A portion of the solid collected from the first crystallization was recrystallized three times from methanol, and the parent amine was regenerated by partitioning between dichloromethane and aqueous (20%) potassium hydroxide solution as described above to yield the pure (−) enantiomer of the diamine: bp 116–119 °C (13 mm); 2.9 g (16 mmol, 18%); $[\alpha]^{20}_D$ (CH_2Cl_2) −92.3°.

Both enantiomers of the diamine were separately acylated with 4-benzo[*b*]thiopheneacetyl chloride as described in method A. The (+) amine (0.20 g, 1.1 mmol) yielded compound **37** (0.27 g, 0.69 mmol, 63%) (the (−) enantiomer of **15**), $[\alpha]^{20}_D$ (CH_2Cl_2) −27°, while the (−) amine (0.20 g, 1.1 mmol) yielded **38** (0.30 g, 0.77 mmol, 70%) (the (+) enantiomer of **15**), $[\alpha]^{20}_D$ (CH_2Cl_2) +30°. Similarly, acylation with 4-benzo[*b*]furanacetyl chloride yielded, from the (+) amine (0.60 g, 3.3 mmol), **39** (0.85 g, 2.0 mmol, 61%) (the (−) enantiomer of **14**), $[\alpha]^{20}_D$ (CH_2Cl_2) −35°, and from the (−) amine (0.40 g, 22 mmol), **40** (0.59 g, 1.6 mmol, 73%) (the (+) enantiomer of **14**), $[\alpha]^{20}_D$ (CH_2Cl_2) +42.5°.

The enantiomeric purity of both of the separated amine enantiomers, **37–40**, were all assayed by using the chiral solvating agent method of Pirkle and Hoover.[29] ^{1}H NMR spectra of equimolar solutions of the compound and (*R*)-(−)-2,2,2-trifluoro-1-(9-anthranyl)ethanol in $CDCl_3$ solution were obtained at 300 mHz. When racemic compounds were used, each resonance in the normal spectrum split into two components, due to the difference between the solvent shifts imparted to each enantiomer by the chiral solvating agent. The most clearly resolved differences were for the two doublets of the AB quartet to Ar CH_2 ($\Delta\delta$ between enantiomers 0.037 ppm for the lower field doublet and 0.053 ppm for the higher field doublet), and for the N*Me* singlet ($\Delta\delta$ between enantiomers, 0.103 ppm). When compounds **37–40** were tested, signals corresponding to only one enantiomer were observed, the opposite enantiomer therefore being absent within the confidence limits of the experiment (>98%) in each case.

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Supplementary Material Available: Crystal data for **27** and tables listing bond lengths and angles, atom coordinates and temperature factors, anisotropic temperature factors, and hydrogen coordinates and temperature factors (9 pages). Ordering information is given on any current masthead page.

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Binaltorphimine-Related Bivalent Ligands and Their κ Opioid Receptor Antagonist Selectivity

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In an effort to develop selective antagonists for κ opioid receptors, bivalent ligands that contain opioid antagonist pharmacophores derived from naltrexone or other morphinans were synthesized and tested on the guinea pig ileum (GPI) and mouse vas deferens (MVD) preparations. The minimum requirements for κ selectivity are at least one free phenolic OH group and one *N*-cyclopropyl or *N*-allyl substituent. Several compounds (**3**, **8**, **10**) with κ selectivity as good as or better than norbinaltorphimine (nor-BNI, **2**) were discovered. The structure–activity relationship revealed that the pyrrole ring functions strictly as a spacer and does not contribute to κ selectivity. The pharmacologic data suggest that only one antagonist pharmacophore may be required for κ selectivity and that the other morphinan portion of the molecule confers selectivity by interacting with a unique subsite proximal to the antagonist pharmacophore recognition locus.

The existence of receptor subpopulations among different receptor classes is now generally recognized. For this reason the advances in many areas of medicinal chemistry and pharmacology depend greatly on highly selective ligands as tools. Since cross-recognition of multiple receptor populations by a ligand may lead to ambiguities in the analysis of structure–activity relationship studies, it is particularly important that highly selective tools are available for this purpose. This is particularly relevant to opioid receptors, as there are at least three major receptor types (μ, κ, δ) in this class.[1]

The opioid antagonists naloxone and naltrexone have been employed extensively as tools in opioid research.[2,3] However, while useful in determining the possible involvement of an opioid mechanism, these antagonists are insufficiently selective to sort out actions mediated by subpopulations of opioid receptors.

In an effort to design highly selective opioid antagonists, we have employed the bivalent ligand approach using a naltrexone-derived antagonist pharmacophore.[4] The term "bivalent ligand" has been given to molecules that contain two recognition units linked through a spacer.[5] The basic assumption was that enhanced potency and selectivity can

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