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## Development of Kappa Opioid Receptor Antagonists

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### Abstract

Kappa opioid receptors (KORs) belong to the G-protein coupled class of receptors (GPCRs). They are activated by the endogenous opioid peptide dynorphin (DYN) and expressed at particularly high levels within brain areas implicated in modulation of motivation, emotion, and cognitive function. Chronic activation of KORs in animal models has maladaptive effects including increases in behaviors that reflect depression, the propensity to engage in drug-seeking behavior, and drug craving. The fact that KOR activation has such a profound influence on behaviors often triggered by stress has led to interest in selective KOR antagonists as potential therapeutic agents. This perspective provides a description of preclinical research conducted in the development of several different classes of selective KOR antagonists, a summary of the clinical studies conducted thus far, and recommendations for the type of work needed in the future to determine if these agents would be useful as pharmacotherapies for neuropsychiatric illness.

### INTRODUCTION

Opioid receptors were discovered in 1973 using opioid radioligand binding assays in brain homogenates.<sup>1–3</sup> Subsequent cloning studies in the early 1990s differentiated three receptors:  $\mu$ ,  $\delta$ , and  $\kappa$ .<sup>4–7</sup> The cDNA clones of  $\kappa$  opioid receptors (KORs) have been isolated and characterized from several species, including humans. The KOR belongs to the G-protein-coupled class of receptors (GPCRs) that are widely expressed throughout the brain and are located in brain areas that are implicated in the modulation of reward, mood state, and cognitive function such as the ventral tegmental area (VTA), nucleus accumbens (NAc), prefrontal cortex (PFC), hippocampus (HPC), striatum (ST), amygdala (AMYG), locus coeruleus (LC), substantia nigra (SN), dorsal raphe nucleus (DRN), and hypothalamus (HL) of both the rat and human brains.<sup>8–11</sup> KORs are activated by the endogenous opioid peptides derived from prodynorphin.<sup>12–14</sup> Even though these peptides bind to the  $\mu$  and  $\delta$  receptors (MOR and DOR, respectively) as well as the KOR, dynorphin A (1–17) (Figure 1) shows preference for the KOR.<sup>15</sup> Upon activation, the KOR couples to the pertussis toxin-sensitive heterotrimeric  $G_{\alpha_{i/o}}$  protein resulting in inhibition of adenylate cyclase, increase in potassium conductance, decrease in calcium conductance, and mobilization of intracellular calcium.<sup>16</sup> In addition, KOR activates the extracellular signal-regulated kinase (ERK 1/2) and p38 mitogen-activated protein kinase (MAPK)<sup>17–19</sup> and can activate c-Jun amino-terminal kinase (JNK).<sup>20</sup> Interestingly, prototypical KOR antagonists can also activate JNK via a pharmacological process called biased agonism or ligand-directed signaling, whereby ligands can inhibit one intracellular signaling pathway while simultaneously activating another.<sup>21,22</sup>

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Relatively early studies showed that activation of the MOR elevates mood, whereas KOR activation produces dysphoria and psychotomimetic effects in humans<sup>23</sup> and anhedonia-, dysphoria-, and anxiety-like effects in rodents<sup>24</sup> (see ref. 25 for a review). These results led to the hypothesis that opposing endogenous opioid systems regulate emotional and perceptual experiences.<sup>23</sup> Figure 2 shows that stimulation of MORs by  $\beta$ -endorphins or a  $\mu$  agonist in the ventral tegmental area increases dopamine (DA) release. In contrast, KOR activation in the nucleus accumbens region by dynorphin (DYN) and  $\kappa$  agonists decreases DA transmission, whereas blockade of the KOR with a  $\kappa$  antagonist increases basal DA release.<sup>26,27</sup> More recent studies have expanded on these early reports. For example, Nestler and Carlezon have proposed that neuroplastic processes that result in enhanced DYN within the NAc promote depressive-like effects in rodents, whereas KOR antagonists have antidepressant-like effects.<sup>28</sup>

While KOR activation resulting from acute stress can facilitate motivation to escape stimuli that represent threats to homeostasis, chronic stress can have adverse effects such as increased risk of depression, increased propensity to participate in drug-seeking behavior, and increased drug-craving.<sup>25</sup> Several studies suggest that DYN activation of the KOR is a key element of these responses. Increased levels of DYN expression are thought to contribute to the negative mood states precipitated by cocaine (1) (Figure 3) withdrawal, a state resembling depression.<sup>29</sup> Rewarding as well as stressful stimuli increase cyclic adenosine monophosphate (cAMP) response element binding protein (CREB) function in the NAc<sup>29,30</sup> (see ref. 31 for a review). The increased levels of dynorphin resulting from activation of CREB seen after stress or drug exposure could contribute to symptoms of emotional numbing.<sup>32</sup> Several studies have established that elevation of CREB function in the NAc elicits the rodent equivalent of signs of major depression.<sup>29,33,34</sup> In addition, the studies showed that stress, a common trigger for addictive as well as depressive disorders, also activates CREB in the NAc.<sup>29,35</sup> In contrast, disruption of CREB function in the NAc had antidepressant-like effects indistinguishable from those of standard antidepressants.<sup>29</sup> The results from these studies suggested that activation of CREB in the NAc is a molecular mediator for aversive or depressive-like symptoms.<sup>29,36,37</sup> Dynorphin released during chronic stress exposure likely produces a variety of depressive-signs in rodents, including immobility in the Porsolt forced-swim test (FST),<sup>38-40</sup> social defeat behavior in the Miczek assay,<sup>41</sup> and potentiation of the rewarding effects of cocaine in the conditioned place preference (CPP) assay.<sup>40</sup> Microinjections of KOR agonists directly into the NAc demonstrate that KOR activation within the region is sufficient to produce anhedonia, a hallmark sign of depressive illness.<sup>42,43</sup>

The fact that KOR function appears to have a profound influence on behaviors that are thought to reflect motivational and emotional states in animal models of depression and anxiety led to interest in the use of selective  $\kappa$  opioid antagonists as potential pharmacotherapies for treating mood disorders.<sup>30,38,44</sup> Initial reports that the prototypical KOR antagonist nor-binaltorphimine (nor-BNI) (2) (Figure 4) produced antidepressant-like effects in the FST in rats<sup>29</sup> were quickly followed up with similar findings in other tests<sup>33</sup> and with (3*R*)-1,2,3,4-tetrahydro-7-hydroxy-N-[(1*S*)-1-[(3*R*,4*R*)-4-(3-hydroxyphenyl)-3,4-dimethyl-1-piperidinyl]methyl]-2-methylpropyl]-3-isoquinolinecarboxamide (JDTic) (3) (Figure 4), a novel, chemically dissimilar KOR antagonist.<sup>38</sup> Additional work showed that KOR antagonists have anxiolytic-like effects and prevent the development of stress-related behavioral adaptations.<sup>45</sup> It has also been shown that KOR antagonists inhibit stress-induced but not cocaine-primed reinstatement of cocaine-associated CPP in mice<sup>46,47</sup> and similarly inhibit stress-induced but not cocaine-primed reinstatement for responding for cocaine in rats.<sup>38</sup> In addition, genetic deletion of the KOR or prodynorphin also abolishes stress-induced reinstatement of CPP in mice.<sup>47</sup> These data clearly suggest that the  $\kappa$  opioid system plays a role in stress-induced cocaine-seeking. Since the relationship between stress and

drug addiction in humans is strong,<sup>48,49</sup>  $\kappa$  opioid antagonists, which enhance stress resilience, also have potential as pharmacotherapies to treat stress-induced relapse to drug seeking. It is important to note that stress is a common factor in these mood- and addiction-related studies, which has led to more broad theories that KOR antagonists may have protective effects against stress.<sup>25,50</sup> Indeed, KOR antagonists can block the aversive and cognitive-disrupting effects of corticotropin-releasing factor,<sup>51–53</sup> a key regulator of stress effects.<sup>54–56</sup> A broad ability to reduce the impact of stress may explain how KOR antagonists can have efficacy in such a wide variety of animal models that would appear to represent different disease states.

### Development of KOR Antagonists

Even though KOR-selective antagonists were initially developed as tools for studying the in vitro and in vivo properties of the KOR agonists, more recent studies have led to the development of KOR antagonists as potential drugs to treat several CNS disorders. In the following sections, the naltrexone-related compounds such as nor-BNI and irreversible binding KOR antagonists which were developed as tools to study the KOR agonists will be briefly summarized, followed by more detailed summaries of several classes of KOR antagonists developed as potential pharmacotherapies to treat mood disorders and drug addiction, as well as to protect against the effects of stress. The status of clinical development of  $\kappa$  opioid antagonist is presented in the Clinical Studies.

### Naltrexone-related Compounds

Even though potent and selective naltrexone (**4**) (Figure 5) related KOR antagonists were discovered in the mid-to late-1980s, they were developed and only used as pharmacological tools to characterize in vitro and in vivo properties of KOR antagonists. An increased understanding of the KOR system as it related to cocaine and opiate abuse as well as mood disorders in the late 1990s and early 2000s led to increased interest in KOR antagonists and their potential to treat human diseases. Since several excellent reviews have covered these early studies, they will not be detailed in this perspective.<sup>57,58</sup> However, a brief summary as it relates to the development of potential pharmacotherapies for treatment of mood disorders, such as depression and anxiety, and addictions, including cocaine, nicotine (**5**), alcohol, and opiates, will be presented.<sup>21,30,31,56,59–65</sup>

The first selective KOR antagonist was the  $\beta$ -naltrexamine derivative 1,8-bis( $\beta$ -naltrexamino)-3,6-dioxaoctane (TENA) (**6**), which was reported by Portoghese and co-workers in 1982.<sup>66</sup> Since its  $\kappa$  selectivity over  $\mu$  and  $\delta$  was only 4- and 2.5-fold, respectively, TENA did not prove to be a very useful compound for studying the KOR. In 1987 Portoghese and co-workers reported the design and development of the naltrexone-derived nor-BNI as the first truly potent and selective KOR antagonist useful for animal studies.<sup>67–70</sup> For example, nor-BNI has selective, centrally mediated  $\kappa$  antagonist effects in mice if the time separating the administration of nor-BNI and the  $\kappa$  agonist is at least 24 h.<sup>70</sup> The resulting  $\kappa$  opioid antagonist activity and selectivity can last up to 28 days.<sup>71,72</sup> Thus, under these conditions nor-BNI is a KOR-selective antagonist.

Portoghese and co-workers also developed 5'-guanidinonaltrindole (GNTI) (**7**) as a more potent and more selective  $\kappa$  opioid antagonist.<sup>73–76</sup> nor-BNI and GNTI have similar features in that they are structural derivatives of the opioid antagonist naltrexone. Like nor-BNI, GNTI also was found to have a slow onset and long duration of action in studies with rhesus monkeys,<sup>76</sup> in fact, GNTI appeared inactive in rats when given by systemic administration, although it had strong behavioral effects in this species when administered by intracerebroventricular (ICV) infusion.<sup>39</sup> The poor bioavailability to GNTI, in particular, has been attributed to specific elements of the molecule (i.e., the high pK<sub>a</sub> of the guanidinium

group).<sup>39</sup> More recent work with nor-BNI in rat demonstrates that the KOR antagonist effects remain detectable for at least 86 days, the time point at which the studies were terminated.<sup>77</sup> Although these types of molecular and pharmacological issues are not understood completely, they have limited enthusiasm for the development of the naltrexone/nor-BNI derivatives as pharmacotherapies. Based on the molecular properties of these compounds, they were not studied as possible pharmacotherapies. Even though a number of nor-BNI analogues have been developed,<sup>57,58,68,75</sup> nor-BNI remains the most widely used KOR antagonist as a pharmacological tool. Some of the seminal studies in which nor-BNI and GNTI were used as pharmacological tools are described below.

Pliakas and co-workers<sup>29</sup> first reported that intracerebroventricularly (i.c.v.) administration of the KOR antagonist nor-BNI decreased immobility in the FST. More thorough analyses that included more doses, drugs (e.g., GNTI, 5'-acetamidinoethylnaltrindole (ANTI) (**8**)), and tests (e.g., intracranial self-stimulation (ICSS)) suggested that these KOR antagonists possess antidepressant-like effects.<sup>39,78</sup> As pointed out in the Introduction, these authors also suggested that their findings are consistent with the hypothesis that CREB-mediated induction of DYN, an endogenous  $\kappa$  agonist<sup>15</sup> in the nucleus accumbens (NAc), triggers immobility behavior in the FST.

A major problem in treating drug addiction is the vulnerability to relapse during abstinence.<sup>79</sup> To gain a better understanding of the mechanisms of relapse, animal models of drug reinstatement were developed to help identify triggers for reinstatement of drug self-administration.<sup>80–83</sup> Drug reinstatement studies in rodents have shown that presentation of drug-associated cues, drug priming, and stress (acute foot shock) each increased drug self-administration.<sup>38,47,84–86</sup> In addition, activation of the  $\kappa$  opioid system by foot shock, forced swim, or KOR agonist 2-(3,4-dichlorophenyl)-*N*-methyl-*N*-[(1*R*,2*R*)-2-pyrrolidin-1-ylcyclohexyl]acetamide (U50,488)<sup>87</sup> (Figure 6) administration reinstated cocaine-seeking behavior in mice.<sup>47</sup> Pretreatment with the selective  $\kappa$  antagonist nor-BNI or by KOR or prodynorphin gene disruption abolishes cocaine reinstatement.<sup>47</sup> Since the relationship between stress and drug addiction is strong,<sup>48</sup> these studies showed that KOR antagonists, which enhance stress resilience, also have potential as pharmacotherapies to treat stress-induced relapse to drug seeking.

There are conflicting results on the use of KOR antagonists for ethanol consumption. Williams and Woods<sup>88</sup> reported no alteration in ethanol consumption following nor-BNI administration in nonhuman primates. nor-BNI also did not alter the alcohol deprivation effects in rats.<sup>89</sup> Mitchell and co-workers<sup>90</sup> reported that treatment with nor-BNI resulted in a significant increase in ethanol consumption in animals with a history of stable ethanol self-administration. In contrast KOR knockout mice showed decreased ethanol drinking.<sup>91</sup> Walker and Koob<sup>92</sup> reported that the effects of nor-BNI on ethanol self-administration vary depending upon whether an animal is naïve or physically dependent. For example, recent work by Walker and co-workers<sup>93</sup> shows that nor-BNI prevents the normal ability of cues that are associated with ethanol withdrawal to trigger ethanol consumption, again suggesting that KOR antagonists can alleviate the impact of aversive or stressful stimuli that contribute to maladaptive (excessive drinking) behaviors.

Forced swim stressed (FSS)-induced potentiation of ethanol reward and self-administration of ethanol is blocked by nor-BNI in mice.<sup>94</sup> Even though Dyn<sup>-/-</sup> mice consumed a similar volume of ethanol as wild-type littermates, they did not demonstrate significant stress-induced increases in consumption. These studies suggest that KOR antagonists may prove beneficial in preventing stress-induced increases in ethanol consumption. nor-BNI (i.c.v. or subcutaneous (s.c.) administration) was also shown to decrease ethanol self-administration in ethanol-dependent animals but had no effect in non-dependent animals.<sup>95</sup> Dependence

was induced using an intermittent alcohol vapor exposure schedule.<sup>95</sup> As shown in the elevated plus maze (EPM) test, male Wistar rats fed on an ethanol liquid diet showed a decrease in the percentage of time spent exploring the open arm and had fewer open arm entries.<sup>96</sup> Pretreatment with nor-BNI attenuated both effects relative to controls. These results are consistent with the hypothesis that the KOR/DYN system is involved in stress-related chronic exposure to ethanol. Even though the results with ethanol are variable, the evidence suggests that KOR antagonist treatment may be effective in the treatment of drug and ethanol addiction.

Smith and co-workers<sup>97</sup> demonstrated that during stress exposure, KOR activation is necessary, and KOR activation in the amygdala alone is sufficient to increase nicotine-seeking behavior as measured by CPP. This increase in nicotine CPP was blocked by the KOR antagonist nor-BNI either by intraperitoneal (i.p.) administration or by local injection in the amygdala without affecting nicotine reward in the absence of stress. Pretreatment with nor-BNI also significantly attenuated stress-induced (forced swim) reinstatement of nicotine-CPP in mice but had no effect on nicotine-primed reinstatement.<sup>98</sup> These results suggest that KOR antagonists will be useful pharmacological tools for studying nicotine addiction and may be useful as pharmacotherapies to prevent relapse to nicotine craving.

It is also interesting to note that cannabinoids and  $\kappa$  opioids act on common elements of the circuitry in the brain and the spinal cord that produce analgesia. For example, antinociception produced by intrathecal administration of the cannabinoid receptor agonist  $\Delta$ -9-tetrahydrocannabinol (THC) (**9**) (Figure 6) and (*R*)-(+)-[2,3-dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo[1,2,3-*de*]-1,4-benzoxazin-6-yl]-1-naphthalenylmethanone (WIN 55,212-2) (**10**) (Figure 6) was attenuated by prior administration of nor-BNI. In contrast, antinociception produced by the non-classical cannabinoid agonist 2-[(1*R*,2*R*,5*R*)-5-hydroxy-2-(3-hydroxypropyl)cyclohexyl]-5-(2-methyloctan-2-yl)phenol (CP 55,940) (**11**) (Figure 6) remained unaffected by prior administration of nor-BNI.<sup>99</sup> Interestingly, nor-BNI also blocked the antinociception produced by spinal administration of THC.<sup>100</sup>

Even though the degree of selectivity and potency following systemic administration of these naltrexone-related ligands was limited, the discovery of opioid-selective antagonists for KORs such as nor-BNI and GNTI by Portoghese and collaborators was of major significance in studies of the relationship to function of opioid receptors. Moreover, they provided valuable information for the design of new antagonists and highlighted the need to develop other selective antagonists.

### Irreversible Binding Compounds

(1*S*,2*S*)-*trans*-2-Isothiocyanato-4,5-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide (–)-UPHIT<sup>101</sup> (**12**) and 2-(3,4-dichlorophenyl)-*N*-methyl-*N*-[(1*S*)-1-(3-isothiocyanatophenyl)-2-(1-pyrrolidinyl)ethyl]acetamide<sup>102,103</sup> (DIPPA) (**13**) (Figure 7) are two irreversible binding ligands that behave like KOR antagonists (see ref. 57 for review). (–)-UPHIT was evaluated for its ability to antagonize the KOR-selective agonist [5*R*-(5 $\alpha$ ,7 $\alpha$ ,8 $\beta$ )]-*N*-methyl-*N*-[7-(1-pyrrolidinyl)-1-oxaspiro[4.5]dec-8-yl]benzeneacetamide (U69,593),<sup>87</sup> the  $\kappa$  agonist/ $\mu$  antagonist brexazocine (**14**), the  $\mu$  agonist [D-Ala<sup>2</sup>, NMe-Phe<sup>4</sup>, Gly-ol<sup>5</sup>]enkephalin (DAMGO) (**15**), and the  $\delta$  antagonist [D-Pen<sup>2</sup>, D-Pen<sup>5</sup>]enkephalin (DPDPE) (**16**)-induced (Figure 8) antinociception in the warm-water tail-flick test after i.c.v. administration.<sup>104</sup> Pretreatment with (–)-UPHIT antagonized U69,593-induced antinociception for up to 48 hours but did not affect brexazocine, the  $\mu$ -selective DAMGO, or the  $\delta$ -selective DPDPE-induced antinociception. These results strongly suggested that (–)-UPHIT is a long-lasting  $\kappa$ -selective opioid antagonist. Similarly, DIPPA produced a long-lasting antagonism of  $\kappa$ -selective agonist ( $\pm$ )-U50,488-induced antinociception in the mouse tail-flick test.<sup>103</sup> DIPPA



also behaved as a mixed agonist/antagonist in the mouse abdominal stretch assay.<sup>103</sup> More recently, DIPPA has been shown to produce anxiolytic-like effects in the novelty-induced hypophagia and defensive burying test in Wistar Kyoto (WKY) and Sprague Dawley (SD) rats.<sup>105</sup> While it is unlikely that these irreversibly binding compounds will become drug candidates, they can serve as useful pharmacologic or biochemical tools to investigate KORs.

### ***N*-Substituted *trans*-3,4-Dimethyl-4-(3-hydroxyphenyl)piperidines**

The *N*-substituted *trans*-3,4-dimethyl-4-(3-hydroxyphenyl)piperidines (**17**) (Figure 9) are a novel class of opioid antagonists where the intrinsic antagonist activity is mediated by the *trans*-3,4-dimethyl orientation of the methyl group on the piperidine ring with the (3*R*,4*R*)-enantiomer being the most potent antagonist.<sup>106–112</sup> All *N*-substituted analogues, including the *N*-methyl analogue (**17**, R = CH<sub>3</sub>), were pure antagonists.<sup>110,111,113,114</sup> As a strategy for obtaining a potent and selective  $\kappa$  opioid selective *N*-substituted *trans*-3,4-dimethyl-4-(3-hydroxyphenyl)piperidine analogue, a library of compounds based on the general structure **18** was synthesized.<sup>115</sup> Evaluation of this library of compounds in inhibition of opioid receptor binding assays identified *N*-{(2'*S*)-[3-(4-hydroxyphenyl)propanamido]-3'-methylbutyl}-(3*R*,4*R*)-dimethyl-4-(3-hydroxyphenyl)piperidine (JPP6) (**19**) as a novel KOR selective ligand.<sup>116</sup> The key structures featured in **19** that provided the  $\kappa$  potency and selectivity were the isopropyl group attached in the (*S*)-configuration for R<sub>1</sub>, a hydrogen substituent for R<sub>2</sub>, and a *p*-hydroxyphenylethyl group for R<sub>3</sub>.<sup>117,118</sup>

Further modification of lead compound **19** led to the discovery of JD*Tic*, a KOR antagonist, with high potency and selectivity in the sulfur-35 guanosine-5'-*O*-(3-thio)triphosphate ([<sup>35</sup>S]GTP $\gamma$ S) *in vitro* functional assay.<sup>117–119</sup> JD*Tic* had a K<sub>e</sub> value of 0.01 nM (pA<sub>2</sub> = 10.46) in the inhibition of U69,593-stimulated [<sup>35</sup>S]GTP $\gamma$ S binding in cloned human KOR.<sup>119</sup> In the same test, nor-BNI had a K<sub>e</sub> = 0.04 nM at the KOR.<sup>120</sup> Similar to nor-BNI, JD*Tic* showed no agonist activity at levels of 10  $\mu$ M and possessed selectivity for the KOR over the MOR and DOR of 341- and 7930-fold, respectively. Using the same *in vitro* assays, nor-BNI had 484- and 113-fold selectivity for the KOR relative to the MOR and DOR, respectively.

A structure-activity relationship (SAR) study revealed that the KOR potency and selectivity exhibited by JD*Tic* result from a combination of (a) the (3*R*,4*R*) configuration of the *trans*-3,4-dimethyl-4-(3-hydroxyphenyl)piperidine core structure; (b) the isoquinoline amino group and 7-hydroxy group held in a rigid orientation by the 1,2,3,4 tetrahydroisoquinoline structure in its 3*R* attachment to the amide carboxyl; (c) an *S*-configuration of the 2-methylpropyl group in the spacer between the piperidine ring and the D-hydroxy Tic acyl group; and (d) the lack of a substituent on the amide nitrogen. More details can be found in Thomas and co-workers.<sup>119</sup>

JD*Tic* was initially evaluated for its ability (a) to antagonize the  $\kappa$  opioid selective agonist enadoline- and the  $\mu$ -selective opioid agonist sufentanil-induced antinociception in ICR male mice;<sup>121</sup> (b) to antagonize the  $\kappa$  selective agonist U50,488-induced antinociception in squirrel monkey using the shock titration procedure;<sup>121</sup> (c) to antagonize U50,488-induced diuresis in SD rats;<sup>121</sup> and (d) to affect the morphine (**20**)-induced physical dependence in adult SD rats.<sup>122</sup>

Pretreatment of mice with s.c. or orally-administered JD*Tic* antagonized effects of enadoline-induced antinociception in the tail-flick test.<sup>121</sup> In contrast to nor-BNI, JD*Tic* has no effect on the  $\mu$  selective agonist sufentanil-induced antinociception in the tail-flick test regardless of the pretreatment time.<sup>121</sup> Unlike nor-BNI, orally administered JD*Tic* also antagonized endoline-induced antinociception. JD*Tic* also potently antagonized the

U50,488-induced antinociception in squirrel monkeys and was more potent than nor-BNI in antagonizing diuresis induced by U50,488 in rats.<sup>121</sup> JD<sub>Tic</sub>, like nor-BNI and GNTI, shows long duration of antagonist activity in these animal tests. Opiate-derived  $\kappa$  antagonist nor-BNI has been reported to potentiate certain overt withdrawal signs in morphine-dependent rats.<sup>123</sup> In contrast, the *trans*-2,4-dimethyl-4-(3-hydroxyphenyl)piperidine derived KOR antagonist JD<sub>Tic</sub> was without significant adverse effect on morphine-induced physical dependence in rats.<sup>122</sup> Importantly, JD<sub>Tic</sub> was able to reduce several symptoms of opiate withdrawal and thus shows potential to be useful for alleviating symptoms of opiate withdrawal in humans.<sup>122</sup>

In the naltrexone-related compounds section, it was pointed out that central administration of nor-BNI and GNTI produced antidepressant-like behavioral effects in the Porsolt rat FST.<sup>29,39</sup> When tested in parallel, there appear to be few differences between the effects of nor-BNI and JD<sub>Tic</sub>, although JD<sub>Tic</sub> may be slightly more potent.<sup>45</sup> For example, JD<sub>Tic</sub> was found to decrease immobility and increase swimming time at doses as low as 0.3 mg/kg (s.c.) in the rat FST, which is consistent with a profile demonstrated by typical antidepressants such as imipramine (lowest active dose was 5.6 mg/kg).<sup>38</sup> Interestingly, these and other KOR antagonists were shown to have anxiolytic-like effects that accompany their antidepressant-like effects.<sup>45</sup> Systemic administration of JD<sub>Tic</sub> and nor-BNI increases open arm exploration in the EPM anxiolytic test and decreases conditioned fear in the fear-potentiated startle paradigm in rats, both anxiolytic-like effects.<sup>45</sup> This is particularly important since KOR antagonists do not possess the reward-related<sup>78</sup> or sedative effects<sup>45</sup> that contribute to the abuse liability of benzodiazepines. The fact that the  $\kappa$  opioid antagonist JD<sub>Tic</sub> and nor-BNI had anxiolytic-like effects that accompanied their antidepressant effects is very interesting since acute administration of serotonin selective uptake inhibitors (SSRIs), which are often used to treat depression and anxiety-related disorders in humans, produce anxiogenic effects.<sup>45</sup> The ability of KOR antagonists to produce acute anxiolytic-like effects together with antidepressant effects differentiates them from standard antidepressants. This behavioral profile suggests that if KOR antagonists were ultimately developed to treat depressive disorders, they might be better tolerated by patients, particularly at the beginning of a treatment regimen.

Depressive disorders are reported to be the most common co-morbid condition among individuals with cocaine abuse disorders, and studies with depressed cocaine abusers report that they have poorer treatment prognosis.<sup>124</sup> Thus, cocaine abusers with depressive symptoms may be especially vulnerable to relapse due to increased cocaine effects. In some studies with imipramine and desipramine, improvement in the reduction of cocaine use was noted.<sup>125,126</sup>

A rat model that addresses relapse to cocaine-taking behavior has been developed. When cocaine is withheld in rats trained to self-administer the cocaine intravenously, they gradually reduce and eventually cease their self-administration behavior. When they are subsequently stressed with foot-shock or primed with a dose of cocaine, reinstatement (relapse) of cocaine-seeking (self-administration) behavior returns. Pretreatment of the rats with the KOR antagonist JD<sub>Tic</sub> using oral administration significantly reduced the stress-induced reinstatement but did not reduce cocaine-primed reinstatement, demonstrating that JD<sub>Tic</sub> specifically targets stress-related effects that regulate relapse to cocaine use.<sup>38</sup>

JD<sub>Tic</sub> was evaluated for its effect on alcohol-seeking behavior, alcohol relapse, and maintenance responding for alcohol in P-rats.<sup>127</sup> Pretreatment with JD<sub>Tic</sub> was effective at decreasing alcohol-seeking and alcohol relapse in P-rats using the Pavlovian Spontaneous Recovery (PSR) and alcohol deprivation models, respectively. However, JD<sub>Tic</sub> did not decrease alcohol maintenance responding in P-rats.<sup>127</sup> In a separate study, both JD<sub>Tic</sub> and

nor-BNI were shown to decrease alcohol self-administration in male Wistar rats.<sup>128</sup> Moreover, pretreatment with JDTC dose dependently reversed acute alcohol withdrawal-induced anxiety (hangover anxiety) and decreased cue-induced reinstatement of alcohol seeking.<sup>128</sup> Surprisingly, pretreatment with JDTC had no effect on stress-induced reinstatement of alcohol seeking in male Wistar rats.<sup>128</sup>

Nicotine is reported to produce a dose-dependent increase in DYN in the striatum<sup>129</sup> and, similar to other substances of abuse, to elevate DA levels in the NAc.<sup>130</sup> In addition, as pointed out in the Introduction, activation of KOR/DYN systems produces aversion, dysphoria, anhedonia, depression, and stress responses in humans and rodent models thought to reflect these effects.<sup>11,23,24,39,78,131,132</sup> Indeed other studies suggested that chronic nicotine exposure changes KOR levels to enhance negative effects.<sup>133</sup> In agreement with these reports, pretreatment with JDTC or nor-BNI blocked spontaneous nicotine withdrawal-induced anxiety-like behavior in the EPM and somatic signs of withdrawal.<sup>134</sup> In addition, both JDTC and nor-BNI blocked the expression of mecamylamine-precipitated nicotine withdrawal.<sup>134</sup>

Disruption in perception and cognition is characteristic of psychiatric conditions such as schizophrenia. The KOR agonist salvinorin A (**21**) and the non-competitive *N*-methyl-D-aspartic acid (NMDA) receptor antagonist ketamine (**22**) are two compounds known to alter perception and cognition in humans. Using the 5-choice serial reaction time task (5CSRTT), a food-motivated test that quantifies attention in rodents, salvinorin A and ketamine were shown to produce the same pattern of disruptive effects characterized by increases in signs often associated with reduced motivation (omission errors) and deficits in processing (elevated latencies to respond correctly).<sup>135</sup> Pretreatment with JDTC blocked all salvinorin A effects and some ketamine effects. Since binding and functional studies revealed that ketamine is a less potent full agonist at KOR, these studies provided evidence that KORs might be involved in some of the cognitive abnormalities observed in psychiatric disorders such as schizophrenia. Thus, modulation of KOR function may represent a unique approach for treating core symptoms of schizophrenia. However, it is also possible to conceptualize the effects of salvinorin A on attention as reflecting a prodepressive- or aversive-like state, especially when considering ketamine's agonist effects at KORs.<sup>135</sup> Work from the Chavkin group has shown that at least some of the aversive effects of KOR agonists are mediated via interactions with corticotropin-releasing factor (CRF).<sup>51</sup> Indeed, it has now been shown that JDTC blocks the cognitive-disrupting effects of CRF,<sup>53</sup> again suggesting interactions between KOR and stress systems that may open the door for new indications for which there are currently no medications.

JDTC also proved useful for obtaining a crystal of the human KOR suitable for X-ray analysis.<sup>136</sup> Elucidation of the high-resolution crystal structure of the human KOR in complex with JDTC will be helpful in the design of new  $\kappa$  antagonists (Figure 11). The representation in Figure 11A shows the large receptor pocket and the tight fit of JDTC into the computer-generated KOR binding pocket. There is a large pocket for the 3,4-dimethyl-4-(3-hydroxyphenyl) ring, a deep pocket for one of the methyls of the isopropyl group, and a flat pocket for the 7-hydroxytetrahydroisoquinoline. The binding diagram in Figure 11B shows 22 contact residues that are within 4.5 Å from the JDTC structure. Salt bridges and hydrogen bonds are shown as red and blue dotted lines, respectively. Residues that vary among MOR, DOR, and KOR are shown in cyan. The conserved Asp 138 is in orange. This crystal structure provides detailed insight into the atomic details of molecular recognition and selectivity of the KOR and provides critical information for structure-based design of new KOR antagonists with improved pharmacological profiles.



In addition to the X-ray structure of JDTC bound to the KOR, the X-ray structures for the MOR, DOR, and ORL-1 receptors bound to inhibitors were reported.<sup>137–139</sup> Knowledge of how inhibitors of the KOR, MOR, DOR, and ORL-1 interact with these receptors will help in the design of better antidepressants, anxiolytics, analgesics, and drugs to treat addiction that lack the undesirable properties of presently available opioid antagonists.

A number of JDTC analogues have been developed which are potent and selective KOR antagonists. Table 1 lists the [<sup>35</sup>S]GTPγS binding data for six of the analogues that have the best KOR efficacy and selectivity.<sup>140,141</sup> All the analogues have sub-nanomolar efficacy for the KOR and greater than 100-fold and 570-fold selectivity relative to the MOR and DOR, respectively.<sup>140,141</sup> Compounds **23a–e** were reported to antagonize U50,488-induced antinociception in a tail-withdrawal test using male C57BL/6 mice<sup>22</sup> and, like JDTC and nor-BNI, **23b** and **23c** had a long duration of action. In contrast, the duration of action for **23a**, **23d**, and **23e** lasted for 1 day or less (Table 1).<sup>22</sup> The effect of **23f** on the antagonism of κ agonist-induced antinociception has not been reported.

Compounds **23c** and **23f** antagonized U50,488-induced diuresis in rats after i.p. administration.<sup>142</sup> In addition, **23c** also blocked U50,488-induced diuresis and prevented footshock-induced reinstatement of cocaine-seeking in rats after oral administration.<sup>142</sup>

### N-Substituted 4β-Methyl-5-(3-hydroxyphenyl)morphans

In a preliminary study, *N*-substituted 4β-methyl-5-(3-hydroxyphenyl)morphans (**24a–i**) were reported to be opioid receptor pure antagonists with [<sup>35</sup>S]GTPγS binding properties similar to those of the *N*-substituted *trans*-3,4-dimethyl-4-(3-hydroxyphenyl)piperidine (**17**) class (Table 2). The morphans **24** can be viewed as conformationally rigid analogues of **17**. A preliminary study identified *N*[(1*R*,4*S*,5*S*,7*R*)-5-(3-hydroxyphenyl)-4-methyl-2-(3-phenylpropyl)-2-azabicyclo[3.3.1]non-7-yl]-3-(1-piperidinyl)propanamide [(–)-KAA1] (**24a**) with a  $K_e = 0.24$  nM at the KOR and 175- and 138-fold selectivity for the κ relative to the MOR and DOR as a potent and selective KOR antagonist.<sup>143</sup> In a follow-up study a number of analogues of **24a** were synthesized where the R and R' groups were varied and evaluated in the [<sup>35</sup>S]GTPγS binding assays.<sup>144</sup> Table 2 lists data for nor-BNI, JDTC, **24a**, and eight of the analogues that showed good potency and selectivity for the KOR. Even though the data on these compounds are currently limited to only [<sup>35</sup>S]GTPγS results, the unique structures of these compounds suggest that physiochemical properties and animal behavioral studies need to be conducted to determine if one or more of these KOR antagonists should be developed.

### Peptides

During the timeframe 1988–2005, a few dynorphin A peptide KOR-selective antagonists were reported, but none of these early developed peptides has been widely studied in animal behavioral test. Four of these peptides are listed below (see ref. 65 for a review).

The peptide in this group that was studied the most was Ac[Phe<sup>1,2,3</sup>,Arg<sup>4</sup>,D-Ala<sup>8</sup>]dynorphin A-(1–11) amide (arodyn) (**28**). Pretreatment with 0.3 or 1 nmol, i.c.v., of arodyn (Figure 12) two hours prior to testing antagonized the antinociceptive effects of U50,488. Pretreatment with 0.3 nmol, i.c.v., of arodyn also prevented stress-induced CPP but not cocaine-induced CPP.<sup>46</sup>

Zyklophin (**29**) (Figure 13) is a cyclic dynorphin A analogue that was developed as the first dynorphin A-based antagonist with modification in the C-terminal “address” domain.<sup>145</sup> Zyklophin had  $K_i$  values of 30.3, 5880, and >10,000 nM at KOR, MOR, and DOR, respectively, using Chinese hamster ovary (CHO) cells stably expressing KOR, MOR, and

DOR.<sup>145</sup> Radioligands [<sup>3</sup>H]diprenorphine, [<sup>3</sup>H]DAMGO, and [<sup>3</sup>H]DPDPE were used for KOR, MOR, and DOR, respectively. For comparison, dynorphin A-(1-11)NH<sub>2</sub> has K<sub>i</sub> values of 0.57, 1.85, and 6.18 at KOR, MOR, and DOR, respectively. Zyklophin also antagonized dynorphin A-(1-13)NH<sub>2</sub> at KOR in an adenylyl cyclase assay with a K<sub>B</sub> = 84 nM.

Pretreatment with zyklophin at doses of 1–3 mg/kg, s.c., or 0.3, 1, or 3 nmol, i.c.v., antagonized the antinociception by the selective KOR agonist U50,488 in C57BL/6J mice tested in the 55 °C warm-water, tail-withdrawal test but had no effect on morphine or SNC-80-mediated antinociception.<sup>146</sup> Zyklophin lacked any antinociception effects after either s.c. or i.c.v. administration. These results suggest that zyklophin selectively antagonized KOR in vivo. A pretreatment dose of 3 mg/kg, s.c., of zyklophin also prevented stress-induced reinstatement of cocaine-seeking behavior in a CPP test. Pretreatment with zyklophin at 1 and 3 mg/kg, s.c., had no effect on cocaine-induced reinstatement of CPP.

Saito and co-workers<sup>147</sup> isolated the cyclic peptide cyclo(Phe-D-Pro-Phe-Trp) (**30**) (CJ-15,208) (Figure 14) from the fermentation broth of a fungus, *Ctenomyces serratus* ATCC15502, and by spectroscopic and chemical means deduced it to be the cyclic tetrapeptide cyclo(Phe-D-Pro-Phe-X<sub>XXX</sub>), where X<sub>XXX</sub> was a tryptophan residue with undefined stereochemistry. Seale and co-workers<sup>148</sup> established that the Trp had the L-configuration, and thus, the structure of **30** was cyclo(Phe-D-Pro-Phe-Trp) by total synthesis.<sup>148</sup> Using a guinea pig brain membrane preparation for binding studies, Saito and co-workers reported that **30** had IC<sub>50</sub> values of 47, 260, and 2600 nM at the KOR, MOR, and DOR, respectively. Thus, **30** has some preference for the KOR.

Compound **30** was reported to be a weak KOR antagonist by showing that **30** reversed the KOR agonist asimadoline-suppressed twitch response in a rabbit vas deferens assay. These interesting studies led two groups to synthesize **30** and to evaluate the compound in different binding and efficacy assays.<sup>149,150</sup> One group,<sup>149</sup> using cloned human, KOR, MOR, and DOR expressed in separate cell lines and [<sup>3</sup>H]diprenorphine as the radioligand, found K<sub>i</sub> = 29, 130, and 2000 for inhibition of binding at the KOR, MOR, and DOR, respectively. The second group,<sup>150</sup> using membranes from CHO cells stably expressing cloned rat KOR and MOR and mouse DOR and [<sup>3</sup>H]diprenorphine, [<sup>3</sup>H]DAMGO, and [<sup>3</sup>H]DPDPE radioligands for KOR, MOR, and DOR, respectively, reported K<sub>i</sub> = 35.4, 619, and 4150 nM for inhibition of KOR, MOR, and DOR binding, respectively. Thus, both groups using different assay conditions confirmed the modest KOR selectivity of **30** reported by Saito and co-workers.<sup>147</sup> However, both groups found that **30** had significantly higher potency for MOR over KOR in [<sup>35</sup>S]GTPγS binding efficacy assay. Dolle and co-workers<sup>149</sup> reported IC<sub>50</sub> = 440 and 25 nM at KOR and MOR, respectively, using human cell lines stimulated by U50,488 and loperamide for KOR and MOR, respectively, and Ross and co-workers<sup>150</sup> reported K<sub>B</sub> = 62.8 and 10.3 at KOR and MOR, respectively, using rat cell lines and dynorphin and DAMGO for stimulation of KOR and MOR.

Both groups<sup>149,150</sup> also synthesized and studied the properties of the D-Trp isomer of **30**, cyclo(Phe-D-Pro-Phe-D-Trp) (**31**) (Figure 14). Dolle and co-workers reported K<sub>i</sub> = 3.8, 30, and >1000 nM for inhibition of radioligand binding at KOR, MOR, and DOR, respectively. Ross and co-workers found K<sub>i</sub> = 30.6, 259, and 2910 nM at KOR, MOR, and DOR, respectively. Even though Dolle and co-workers found that **31** had higher efficacy at KOR than **30**, both groups reported that **31** had modest KOR selectivity similar to that of **30**. In contrast, the two groups reported quite different results for the [<sup>35</sup>S]GTPγS binding assay of **31**. Using the [<sup>35</sup>S]GTPγS binding assay, Dolle and co-workers reported IC<sub>50</sub> values of 140 and 21 nM at KOR and MOR, respectively, IC<sub>50</sub> values similar to those for **30**. Ross and co-workers found K<sub>B</sub> values of 22.8 for KOR and no antagonist efficacy at 300 nM at MOR in

[<sup>35</sup>S]GTPγS assays. This difference could be due to the fact that Dolle and co-workers used human cell lines, whereas Ross and co-workers used rat cell lines. A recent study has shown that there can be differences between human and rat KORs.<sup>151</sup> However, additional studies will be needed to explain the different results. In their study Dolle and co-workers found that cyclo(Ala-D-Pro-Phe-D-Trp) (**32a**) (Figure 14) was a dual KOR/MOR antagonist with 10-fold greater in vitro functional efficacy (IC<sub>50</sub> = 5 nM, KOR; 48 nM, MOR) relative to cyclo(Phe-D-Pro-Phe-D-Trp) (**31**). Dolle and co-workers also were able to obtain an X-ray crystal structure of **32a**, which showed that at least this structure might yield a suitable complex with the human KOR suitable for analysis. In more recent studies Aldrich and co-workers<sup>152</sup> reported that cyclo(Ala-D-Pro-Phe-Trp) (**32b**) was also a dual KOR/MOR antagonist with K<sub>B</sub> = 2.6 and 7.3 nM at KOR and MOR, respectively, in a [<sup>35</sup>S]GTPγS assay. However, **32b** exhibited antinociceptive potencies (ED<sub>50</sub> = 1.49 nM, i.c.v.) in the warm-water, tail-withdrawal test similar to that of **30** which was almost completely blocked by the MOR-selective irreversible antagonist β-funaltrexamine (β-FNA). Thus, even though **32b** showed no agonist efficacies in the [<sup>35</sup>S]GTPγS assay, it was a potent MOR agonist in the warm-water tail withdrawal test.

Ross and co-workers<sup>150</sup> evaluated **31** in the warm-water, tail-withdrawal assay using C57B1/6J mice. Pretreatment of **31** with 3-nmol, i.c.v., doses 80 min before agonist administration significantly antagonized the antinociception effects of the KOR-selective agonist U50,488 but not of the MOR and DOR agonist morphine and SNC-80, respectively. The D-Trp isomer of **30** also had about 10% antinociceptive activity at an i.c.v. dose of 10 nmol. Somewhat surprisingly, **30** had an EC<sub>50</sub> = 3.71 nmol as an agonist in the antinociceptive test. The results suggested that the compound acted as a combined KOR and MOR receptor agonist. Thus, **30** was a selective antagonist of U50,488-induced antinociception but with weaker potency than that of **31**.

Compound **31** was also evaluated in a CPP model of stress-induced reinstatement of cocaine-seeking behavior using conditions similar to those used for zyklophin. Pretreatment (daily for 2 days) with **31** (3 nmol, i.c.v.) prevented stress-induced reinstatement of cocaine place preference. However, pretreatment with **31** (3 mmol, i.c.v.) was ineffective at blocking cocaine-induced reinstatement.

### Amidosulfonylbiphenylamines

Using a high throughput screening process, a group from Pfizer PharmaTherapeutics Research and Development identified two compounds with K<sub>i</sub> values for inhibition of [<sup>3</sup>H]diprenorphine of CHO cells expressing KOR and MOR. The first compound **33** (Figure 15) had a K<sub>i</sub> = 2.49 nM and 1.60 nM at KOR and MOR, respectively.<sup>153</sup> The second compound **34** had K<sub>i</sub> = 9 and 21 nM for KOR and MOR, respectively. Compound **33** was a functional agonist in the [<sup>35</sup>S]GTPγS binding assay, whereas **34** was a functional antagonist. Lead optimizations of **33** and **34** to improve KOR potency and selectivity relative to the MOR as well as improve drug-like properties led to the selection of 2-methyl-*N*-((2'-(pyrrolidin-1-ylsulfonyl)biphenyl-4-yl)methyl)propan-1-amine (PF-04455242) (**35**) for preclinical development.<sup>154</sup> Compound **35** has K<sub>i</sub> values of 3 and 65 nM in a radioligand binding assay using CHO cell membranes expressing human KOR and MOR, respectively. Thus, **35** has 21-fold selectivity for κ relative to μ. The compound has negligible affinity for DOR in the human DOR (K<sub>i</sub> = >4 μM). Compound **35** was reported to have K<sub>i</sub> antagonist values of 1.23 and 10 nM at the KOR and MOR, respectively, using a [<sup>35</sup>S]GTPγS binding assay. The assay used CHO cell membranes expressing the human KOR and MOR and U50,488 and morphine for stimulation of the KOR and MOR, respectively. When tested at 100 nM, **35** has no agonist activity. The physicochemical properties—P-glycoprotein (P-gp) liability and human liver microsomal clearance—for **35** were improved over those for the

lead structures **33** and **34**.<sup>153</sup> Ex vivo and in vivo radioligand binding studies in rats were used to show that **35** had significantly higher KOR occupancy compared to MOR and to help establish the dose for use in animal behavioral studies.

Using male ICR mice and the procedure reported by D'Amour and Smith,<sup>155</sup> **35** was shown to antagonize the effects of U50,488 or morphine with AD<sub>50</sub> values of 0.67 and 12.03 mg/kg, respectively.<sup>153</sup> In a rat tail-flick test **35** had AD<sub>50</sub> values of 1.5 and 9.8 mg/kg for antagonist of  $\kappa$  and  $\mu$  agonist-induced antinociception. Compound **35** at 10 and 32 mg/kg did not have any agonist effect in mice on latency to tail withdrawal. Pretreatment with **35** (1 h, s.c.) reduced immobility in mice with a minimal effective dose of 3.2 mg/kg in the FST and was also active in the social deficit stress assay.<sup>154</sup> Pretreatment with **35** attenuated stress-induced reinstatement of cocaine CPP in mice but did not demonstrate rewarding or aversive effect directly.<sup>154</sup>

### Aminobenzoyloxyarylamides

In a study directed toward preclinical development of an opioid receptor occupancy tracer, some interesting KOR antagonists were identified.<sup>156</sup> Radioligand binding studies using [<sup>3</sup>H]diprenorphine and membranes from CHO cells expressing human  $\kappa$  and  $\mu$  or HEK293 cells expressing DORs, respectively, showed that 4-{4-[2-(3,5-dimethylphenyl)pyrrolidin-1-ylmethyl]phenoxy}-3-fluorobenzamide (LY2456302) (**36a**) (Figure 16) had a K<sub>i</sub> value of 0.949 at the KOR and was 24- and 175-fold selective for the KOR relative to the MOR and DOR, respectively.<sup>156</sup>

In an in vitro inhibition of agonist stimulants, [<sup>35</sup>S]GTP $\gamma$ S binding study using membranes from CHO cells expressing human KOR and MOR and KOR-selective U69,593 and MOR-selective agonist DAMGO, respectively, for stimulation and using membranes from HEK293 cells expressing the human DOR and selective  $\delta$  agonist DPDPE, **36a** had a K<sub>e</sub> = 0.813 with selectivity of 21- and 135-fold, respectively.<sup>156</sup> Compounds **36b** and **36c** (Figure 16) with K<sub>i</sub> values of 0.722 and 0.565 nM, respectively, were slightly more potent than **36a**. Compound **36b** was 36- and 212-fold selective for KOR relative to MOR and DOR, respectively. Compound **36c** with a 63- and 373-fold selectivity for KOR over MOR and DOR, respectively, was the most KOR-selective compound in the radioligand binding assay. Compounds **36b** and **36c** had K<sub>b</sub> values at the KOR of 0.632 and 1.57 nM, respectively, which are slightly lower and larger, respectively, than the value for **36a**. Compounds **36b** and **36c** have 132- and 187-fold selectivity for KOR over DOR, respectively, but like **36a** have very low (11- and 14-fold selectivity) relative to MOR. Enantiomers **37a–c** of **36a–c** (Figure 16) showed much lower KOR potency in both the radioligand binding and the [<sup>35</sup>S]GTP $\gamma$ S assays.

Compound **36c** antagonized the antinociceptive activity of 1 mg/kg, s.c., of the KOR-selective agonist U69,593 with an ED<sub>50</sub> = 0.24 mg/kg, orally (p.o.). Compound **36c** had an ED<sub>50</sub> of 30 mg/kg for antagonizing a 10-mg/kg, s.c., dose of morphine. Thus, even though **36c** has very low selectivity for KOR relative to MOR in vitro, it is KOR-selective in the rat formalin test.

In a separate study,<sup>157</sup> new K<sub>b</sub> values were determined for **36a** using the [<sup>35</sup>S]GTP $\gamma$ S functional assay in cloned human MOR, DOR, and KOR. For antagonist assays, DAMGO, DPDPE, and U69,593 were used for stimulation of MOR, DOR, and KOR, respectively. The K<sub>b</sub> values found for **36a** were 40.1, 2.12, and 264 nM at the MOR, KOR, and DOR, respectively. In vitro binding using high guanosine 5'-diphosphate sodium salt (Na<sup>+</sup>/GDP), K<sub>i</sub> values for **36a** were given as 16.4, 122, and 0.597 nM for MOR, DOR, and KOR, respectively. Using a liquid chromatography-tandem mass spectrometry (LC/MS/MS)

procedure, **36a** was shown to have good KOR occupancy ( $\kappa$  opioid receptor occupancy (KRO)) in rat brain in the striatum. No significant occupancy of either  $\mu$  or  $\delta$  receptor (measured in the striatum) at doses up to 30 mg/kg was demonstrated. Thus, the ED<sub>50</sub> is greater than 30 mg/kg at  $\mu$  and  $\delta$  receptors.

Compound **36a** at doses of 1 and 3 mg/kg, p.o., decreased the amount of ethanol consumed in an ethanol-drinking maintenance test using female P rats.<sup>157</sup> Using a female P rat progressive ratio responding for ethanol test, pretreatment with **36a** at 10 mg/kg, p.o., reduced the number of active lever responses, and the quantity of ethanol consumed and the breakpoint were also reduced. Results from this study were evidence of a reduction in motivation to work for access to ethanol despite their extensive operant history for ethanol.<sup>157</sup>

Compound **36a** was evaluated in the mouse FST.<sup>157</sup> Similar to the rat FST, this test measures immobility of mice after pretreatment with test compounds. The less immobility compared to vehicle, the more pronounced the antidepressant-like effect. At 10 mg/kg, p.o., **36a** showed a decrease in immobility relative to vehicle but did not show a decrease at 1 and 3 mg/kg, p.o.<sup>157</sup>

Combined doses of **36a** and imipramine were also evaluated in the mouse FST.<sup>157</sup> An inactive 3-mg/kg, p.o., dose of **36a** combined with an inactive 5-mg/kg, i.p., dose of imipramine was as efficacious as an active 15 mg/kg, i.p., of imipramine alone relative to vehicle, imipramine 5 mg/kg, i.p., alone and 3 mg/kg, p.o. of **36a** alone. In addition a 1-mg/kg, p.o., dose of **36a** combined with a 5-mg/kg, i.p., dose of imipramine decreased immobility relative to vehicle control, imipramine 5 mg/kg, i.p., alone and 1 mg/kg, p.o., of **36a** alone. These results suggest that the decrease in immobility of **36a** and imipramine operate through different mechanisms.

Compound **36a** was evaluated for its reversal of U69,593-induced and morphine-induced prepulse inhibition (PPI).<sup>157</sup> PPI is a neurological phenomenon in which a weaker prestimulus (prepulse) inhibits the reaction of an animal (or human) to a subsequent strong startling stimulus (pulse). Compound **36a** reverses the sensory motor gating deficiencies induced by U69,593 and morphine in male Sprague Dawley rats.

### 8-Azabicyclo[3.2.1]octan-3-yloxybenzamides

Through the process of screening their corporate compound collection, a group at AstraZeneca Pharmaceuticals identified **38** (Figure 17) as a structure for the development of a KOR antagonist. Compound **38** had a  $K_i = 0.5$  nM at the KOR for inhibition of radioligand binding and an IC<sub>50</sub> = 77 nM for functional antagonism at the KOR using the [<sup>35</sup>S]GTP $\gamma$ S assay. Functional antagonism at the MOR and DOR were >30  $\mu$ M. An SAR study led to 3-[[[(3-endo)-8-[(5-methyl-2-thienyl)methyl]-8-azabicyclo[3.2.1]oct-3-yl]oxy]-benzamide (AZ-MTAB) (**39**) (Figure 17) which had IC<sub>50</sub> values of 20, 722, and 8306 nM at KOR, MOR, and DOR, respectively, in the [<sup>35</sup>S]GTP $\gamma$ S functional assays.

The AstraZeneca compound **39** was compared to the Eli Lilly compound **36a**, nor-BNI, and JDtic for their ability to antagonize a 2.5-mg/kg, s.c., dose of U50,488-induced diuresis. All four compounds dose-dependently blocked U50,488-induced diuresis on the initial test (day 0, 5-h urine collection). As previously reported,<sup>158</sup> nor-BNI and JDtic (no additional administration) also antagonized a 2.5-mg/kg, s.c., dose of U50,488 on day 7.

The AstraZeneca compound **39** and the Eli Lilly compound **36a** were evaluated for anxiolytic-like activity in prenatal-stress rat using the EPM test. This test is based on evidence that the effects of early-life stress can be persistent into adulthood and can lead to



mood disorders,<sup>159</sup> a test that can be used to model anxiety and depression.<sup>160,161</sup> The test begins by stressing female rats for 7 days that comprise the third trimester. The off-spring of these stressed rats were raised to adulthood and then tested for anxiety-like behavior in the EPM. Prenatal stress suppressed percentage time in the open arms and open arm entries. Prenatal stressed rats dosed 2 h prior to testing in the EPM with **39** (30  $\mu\text{mol/kg}$ ) and **36a** (24  $\mu\text{mol/kg}$ ) increased time spent in open arms and open arms entries that were suppressed by prenatal stress.

## Clinical Studies

There is some clinical evidence that non-selective KOR antagonists may be useful for treating depression. In a double-blind study the  $\kappa$  antagonist partial  $\mu$  agonist buprenorphine (**40**) (Figure 18) induced antidepressant effects in patients with endogenous depression.<sup>162</sup> In addition, Kosten and co-workers<sup>163</sup> reported that depressive symptoms were significantly decreased with buprenorphine treatment in heroin (**41**)-addicted patients who were depressed at intake.<sup>163</sup> Buprenorphine was also shown to be effective in treatment of refractory depression.<sup>164</sup>

In initial human studies using opiate-dependent patients, Kosten and co-workers reported that patients maintained on buprenorphine showed a greater reduction in cocaine use than those on methadone<sup>165,166</sup> (**42**). Even though a larger-scale comparison of buprenorphine to methadone did not show significant superiority of buprenorphine to methadone,<sup>163</sup> the initial studies were suggestive that reduction in cocaine use seen in the initial studies could be due to the KOR antagonist properties of buprenorphine.

As a way to isolate the  $\kappa$  antagonism properties of buprenorphine without concomitant  $\mu$  partial agonist properties, combinations of buprenorphine and the  $\mu/\kappa$  antagonist naltrexone were studied (see ref. 167 for a review). Rothman and co-workers<sup>168</sup> was the first to study this combination. These authors reasoned that the high relapse rate of opiate-dependent patients on withdrawal from methadone was due to the existence of a protracted abstinence syndrome, characterized by a dysphoric mood. They suggested that increases in brain dynorphin levels might be functioning as a homeostatic system to oppose the mood-enhancing and reinforcing effects, an effect opposite to that of morphine as depicted in Figure 2 in the Introduction. These authors hypothesized “that  $\kappa$  overdrive may contribute to the dysphoric mood observed in the protracted abstinence syndrome and that a KOR antagonist might help prevent relapse in abstinent opioid-dependent patients.”<sup>169,170</sup> Since there was no clinically available selective KOR antagonist to use, Rothman and co-workers used a combination of buprenorphine with naltrexone to obtain a functional KOR antagonist absent of  $\mu$  partial agonist activity for their clinical test study. The study showed a substantial reduction in cocaine and opiate use during a three-month trial.<sup>168</sup>

In a more recent open-label study, Gerra and co-workers<sup>171</sup> showed that a combination of buprenorphine and naltrexone significantly enhanced compliance and drug abstinence from heroin and cocaine compared with naltrexone alone in heroin-dependent patients. Similar to the study by Rothman and co-workers,<sup>168</sup> these results were postulated to be attributable to less aversive responses using the buprenorphine/naltrexone combination compared with naltrexone alone. Both the Rothman<sup>168</sup> and Gerra<sup>171</sup> studies support the hypothesis of the therapeutic potential of KOR antagonist in drug addiction.

In a recent review, McCann suggested that a buprenorphine/naltrexone combination might also be useful for treating alcohol, methamphetamine, and nicotine dependence.<sup>167</sup> In addition, McCann suggested that a buprenorphine/naltrexone combination might be useful for patients using various combinations of drugs of abuse such as cocaine and alcohol.<sup>167</sup> Since buprenorphine has NOP (nociceptin receptor) agonist activity, McCann pointed out

that the beneficial effects of the buprenorphine/naltrexone combination might be due in part to this NOP agonist activity.<sup>167</sup>

At this point no selective KOR antagonist has been tested for treatment of mood disorders or drug abuse. However, Phase I clinical studies have been completed for the Eli Lilly compound **36a**, JD<sub>T</sub>ic, and the Pfizer compound **35**. Phase I studies with JD<sub>T</sub>ic<sup>172</sup> and the Pfizer compound<sup>173</sup> (**35**) were terminated; in the case of JD<sub>T</sub>ic, the reason for termination is still undergoing analysis, and Pfizer has not yet shared the reason for termination of their studies. The Phase I clinical study with **36a** was to assess the KOR occupancy after single oral administration of **36a** as measured by positron emission tomography (PET) with PET imaging radioligand LY2879788 in healthy subjects with the condition listed as alcohol dependence.<sup>174</sup> The last update of the study was May 5, 2011. No new studies for **36a** are listed in ClinicalTrials.gov.

## Summary and Perspective

The hypothesis that KOR antagonists might have use for the treatment of depression and drug abuse developed from molecular pharmacology and animal behavioral model studies demonstrating that stress or repeated exposure to drugs of abuse can activate the KOR system via the endogenous ligand dynorphin which can be blocked by KOR antagonists.

Numerous studies have shown that activation of the KOR/DYN system plays a major role in stress-induced reinstatement of drug seeking. Moreover, KOR function has a profound influence on behaviors that reflect motivational and emotional states in animal behavioral models. Blockade of KOR receptors with selective KOR antagonists is a new strategy for protecting individuals from relapse to drug addiction. In addition, KOR antagonists may have potential for treatment of psychiatric disorders, especially those that are produced or exacerbated by stress. Studies showed that KOR antagonists attenuate stress-induced reinstatement of cocaine-seeking in rats<sup>38</sup> and stress-induced cocaine CPP in mice.<sup>40</sup> These studies suggested that activation of the KOR/DYN leads to depression and cocaine relapse, and the decreases of the KOR system may be effective treatments of depression and drug abuse.

It has been difficult to evaluate the hypothesis that KOR antagonists would have therapeutic effects in clinical efficacy trials because of limitations with the currently available compound, as described above. However, during the last few years, there has been increased interest in the KOR/DYN system, and more effort has been directed toward the development of improved selective KOR antagonists. The studies have provided information showing that various scaffolds can lead to potent and selective KOR antagonists. In addition, studies with these compounds have provided additional information concerning their activities in various animal behavioral models of mood disorders and drug abuse. This information is reviewed in this perspective along with the encouraging results that three KOR antagonists—JD<sub>T</sub>ic, the Pfizer compound **35**, and the Eli Lilly compound **36a**—have reached Phase I clinical studies. In addition, studies using a buprenorphine/naltrexone combination to generate a functional KOR antagonist have given promising results in patients addicted to cocaine. It was recently reported at a conference that another propriety formulation that reveals the KOR antagonist effects of buprenorphine has antidepressive effects in humans.<sup>175</sup> These studies are broadly consistent with preclinical studies in laboratory animals suggesting that KOR antagonists have untapped potential as therapeutic agents despite the current lack of a first-in-class lead molecule.

Even though much has been learned from studies with the new KOR antagonists, considerable research will be required before the details of the mechanisms giving these

effects are fully understood. More information about the part that CRF, P-38 MAPK, serotonin, norepinephrine, and the hypocretin/orexin system will provide new insights into how the KOR regulates mood states and drug addiction. In addition, research to determine how CREB regulates stress-induced reinstatement of drug seeking, depression, and sensitivity to both reward and aversion needs to be pursued, particularly to its modulation of DYN and KOR activation.

Even though a few better drug-like KOR antagonists than nor-BNI (2) and GNTI (7) have been developed, new KOR antagonists possessing novel scaffolds are still needed both as potential pharmacotherapies and as pharmacological tools to better understand the KOR/DYN system. Considering that most of the studies have been conducted with prototypical KOR antagonists, all of which have long (or irreversible) durations of action despite apparent chemical dissimilarities, the availability of non-peptidergic short-acting compounds will provide crucial insight into the intracellular consequences of these agents that might lead to more effective high-throughput screening (HTS) procedures. In this regard, a recent study involving a broad range of KOR-blocking agents found a positive correlation between duration of action and JNK activation.<sup>22</sup> While not yet broadly implemented, it is conceivable that secondary screens could be utilized to quantify JNK activation in early hits from HTS procedures to identify optimal chemical scaffolds that serve as the basis for short-acting KOR antagonists. One potential complication is that the biased agonist effects of KOR antagonists have been most thoroughly characterized using rodents, and there is evidence of species differences in KOR function.<sup>151</sup> A long duration of action may ultimately prove to be a desirable characteristic once a drug has been proven to be both safe and efficacious; indeed, the development of sustained-action formulations is common for psychiatric medications (e.g., Buprenorphine SR<sup>TM</sup>).

The elucidation of the high-resolution crystal structure of the human KOR in complex with JD1c will be helpful in the design of new  $\kappa$  antagonists (Figure 11). This crystal structure provides detailed insight into the atomic details of molecular recognition and selectivity of the KOR and provides critical information for structure-based design of new KOR antagonists with improved pharmacological profiles. Regardless, the process of developing KOR antagonists into medications for neuropsychiatric illness has strong origins in preclinical discoveries of how the brain encodes states such as stress, anhedonia, and dysphoria.

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## ABBREVIATIONS

<b>KOR</b>	$\kappa$ opioid receptor
<b>GPCRs</b>	G-protein-coupled receptors
<b>VTA</b>	ventral tegmental area
<b>NAc</b>	nucleus accumbens
<b>PFC</b>	prefrontal cortex
<b>HPC</b>	hippocampus
<b>ST</b>	striatum

<b>AMYG</b>	amygdala
<b>LC</b>	locus coeruleus
<b>SN</b>	substantia nigra
<b>DRN</b>	dorsal raphe nucleus
<b>HL</b>	hypothalamus
<b>ERK</b>	extracellular signal-regulated kinase
<b>MAPK</b>	mitogen-activated protein kinase
<b>JNK</b>	c-Jun amino-terminal kinase
<b>MOR</b>	$\mu$ opioid receptor
<b>DYN</b>	dynorphine
<b>DA</b>	dopamine
<b>cAMP</b>	cyclic adenosine monophosphate
<b>CREB</b>	cAMP response element-binding protein
<b>FST</b>	forced-swim test
<b>CPP</b>	conditioned place preference
<b>nor-BNI</b>	norbinaltorphimine
<b>JDTic</b>	(3 <i>R</i> )-1,2,3,4-tetrahydro-7-hydroxy-N-[(1 <i>S</i> )-1-[[ <i>(3R,4R)</i> -4-(3-hydroxyphenyl)-3,4-dimethyl-1-piperidinyl]methyl]-2-methylpropyl]-3-isoquinolinecarboxamide
<b>TENA</b>	1,8-bis( $\beta$ -naltrexamino)-3,6-dioxaoctane
<b>GNTI</b>	5'-guanidonaltindole
<b>i.c.v</b>	intracerebroventricularly
<b>ANTI</b>	5'-acetamidinoethylnaltindole
<b>ICSS</b>	intracranial self-stimulation
<b>FSS</b>	forced-swim stress
<b>s.c</b>	subcutaneous
<b>EPM</b>	elevated plus maze
<b>i.p</b>	intraperitoneal
<b>THC</b>	$\Delta^9$ -tetraacannabinoid
<b>(-)-UPHIT</b>	(1 <i>S</i> ,2 <i>S</i> )- <i>trans</i> -2-isothiocyanato-4,5-dichloro- <i>N</i> -methyl- <i>N</i> -[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide)
<b>DIPPA</b>	2-(3,4-dichlorophenyl)- <i>N</i> -methyl- <i>N</i> -[(1 <i>S</i> )-1-(3-isothiocyanatophenyl)-2-(1-pyrrolidinyl)ethyl]acetamide
<b>DAMGO</b>	[D-Ala <sup>2</sup> , NMe-Phe <sup>4</sup> , Gly-ol <sup>5</sup> ]enkephalin
<b>DPDPE</b>	[D-Pen <sup>2,5</sup> ]Enkephalin, [D-Pen <sup>2</sup> ,D-Pen <sup>5</sup> ]enkephalin
<b>WKY</b>	Wistar Kyoto
<b>SD</b>	Sprague Dawley

<b>JPP6</b>	<i>N</i> -{(2′ <i>S</i> )-[3-(4-hydroxyphenyl)propanamido]-3′-methylbutyl}-(3 <i>R</i> ,4 <i>R</i> )-dimethyl-4-(3-hydroxyphenyl)piperidine
<b>[<sup>35</sup>S]GTPγS</b>	sulfur-35 guanosine-5′- <i>O</i> -(3-thio)triphosphate
<b>SAR</b>	structure-activity relationship
<b>SSRI</b>	serotonin selective uptake inhibitor
<b>PSR</b>	Pavlovian Spontaneous Recovery
<b>NMDA</b>	<i>N</i> -methyl-D-aspartic acid
<b>CRF</b>	corticotropin-releasing factor
<b>5CSRTT</b>	5-choice serial reaction time task
<b>KAAl</b>	<i>N</i> -[(1 <i>R</i> ,4 <i>S</i> ,5 <i>S</i> ,7 <i>R</i> )-5-(3-hydroxyphenyl)-4-methyl-2-(3-phenylpropyl)-2-azabicyclo[3.3.1]non-7-yl]-3-(1-piperidinyl)propanamide
<b>DOR</b>	δ opioid receptor
<b>CHO</b>	Chinese hamster ovary
<b>P-gp</b>	P-glycoprotein
<b>p.o</b>	by mouth
<b>Na<sup>+</sup>/GDP</b>	guanosine 5′-diphosphate sodium salt
<b>LC/MS/MS</b>	liquid chromatography-tandem mass spectrometry
<b>KRO</b>	κ opioid receptor occupancy
<b>PPI</b>	prepulse inhibition
<b>PET</b>	positron emission tomography
<b>NOP</b>	nociceptin receptor

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## Biographies

**F. Ivy Carroll, Ph.D.** received the B.S. degree in chemistry from Auburn University in 1957 and was awarded the Ph.D. in chemistry by the University of North Carolina at Chapel Hill in 1961. He joined the research staff of the Research Triangle Institute as a Research Chemist and rose steadily to the position of Vice President of the Chemistry and Life Sciences Group, a position he held from 1996–2001. Dr. Carroll also served as Director of the Center for Organic and Medicinal Chemistry from 1975–2007. He is presently Distinguished Fellow for Medicinal Chemistry. Dr. Carroll has varied research interests, but since 1990, a major thrust of his research efforts involved development of pharmacotherapies for substance abuse (cocaine, nicotine, methamphetamine, opioids, and ethanol) and other CNS disorders.

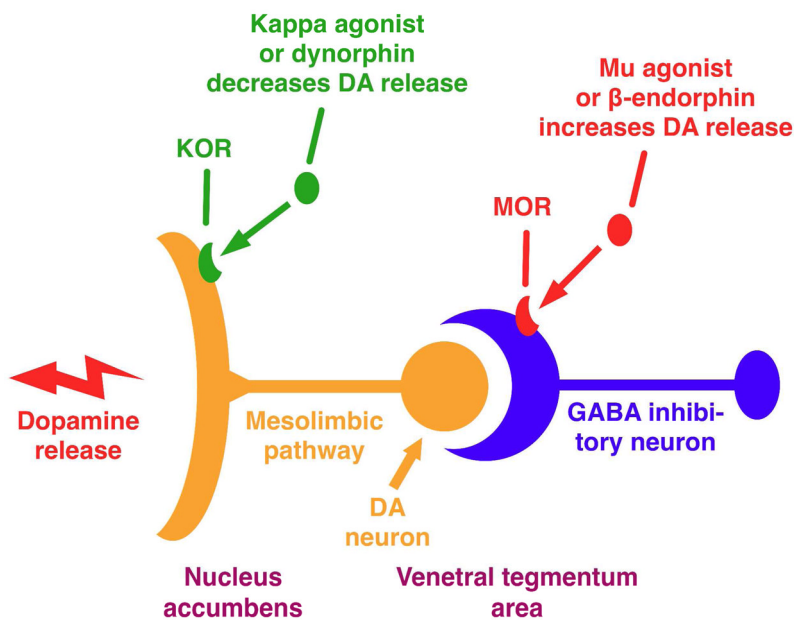
**William A. Carlezon, Jr., Ph.D.** is a Professor of Psychiatry at Harvard Medical School, Director of the Behavioral Genetics Laboratory at McLean Hospital, and Editor-in-Chief of *Neuropsychopharmacology*. His research focuses on nature/nurture issues as they relate to the brain. He and his team study the basic processes by which the brain develops and is modified in response to experience, including exposure to stress, drugs, trauma, or toxins. His work is applicable to a variety of psychiatric and neurologic conditions, including stress-related disorders, addiction, and autism.



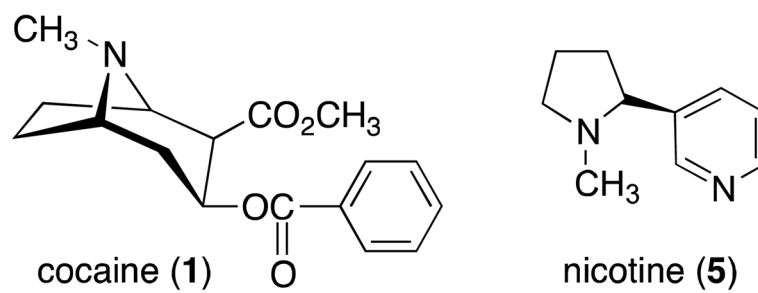
Tyr - Gly - Gly - Phe - Leu - Arg - Arg - Ile - Arg -

Pro - Lys - Leu - Lys - Trp - Asp - Asn - Gln - OH

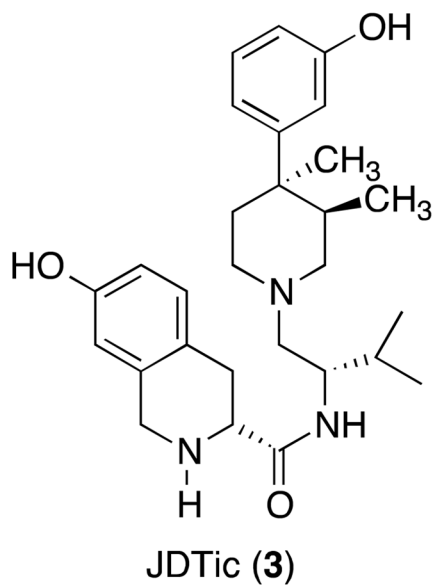
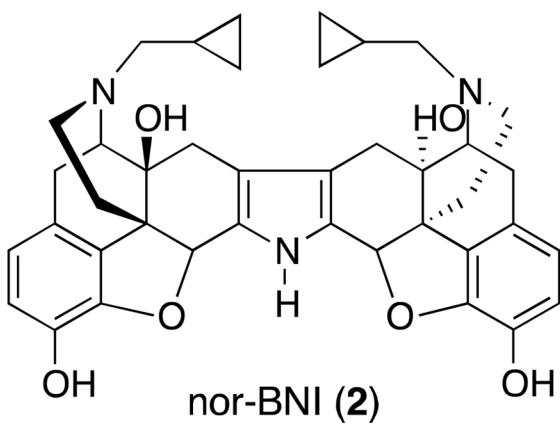
**Figure 1.**  
Structure of dynorphin A (1–17) amino acid abbreviation YGGFLRRIRPKLKWDNQ



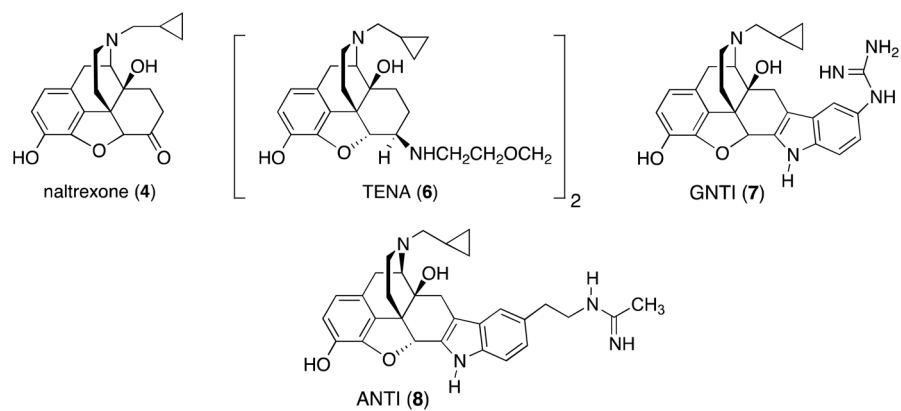
**Figure 2.**  
Model for modulating the endogenous opioid system<sup>26</sup>



**Figure 3.**  
Structures of cocaine and nicotine

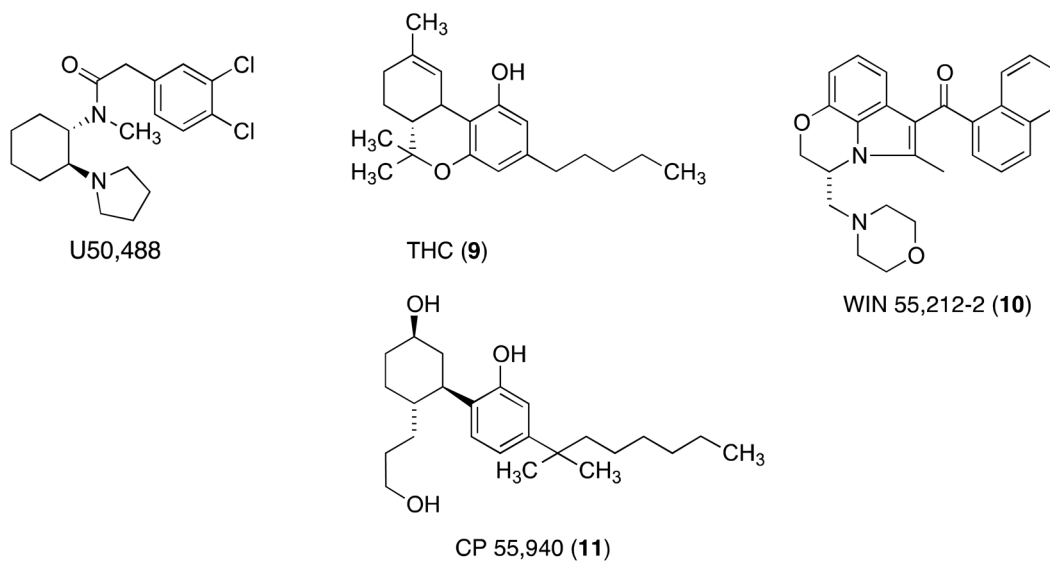


**Figure 4.**  
Structures of nor-BNI and JDTic

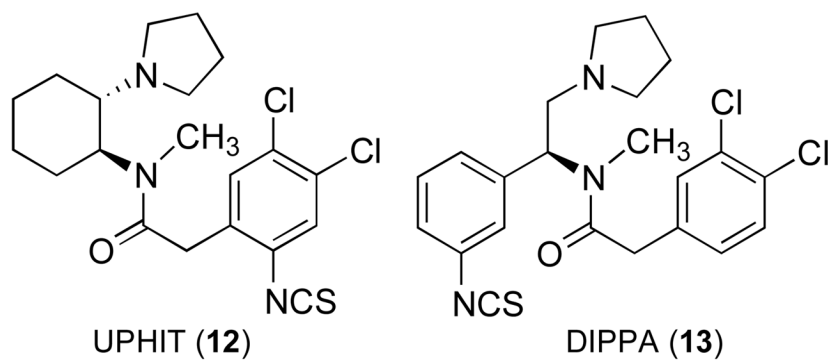


**Figure 5.**  
Structures of naltrexone, TENA, GNTI, and ANTI

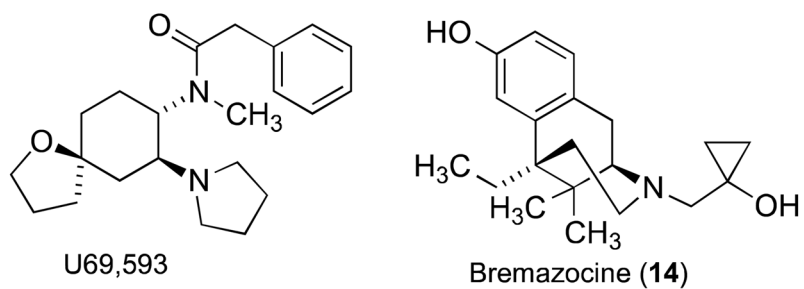




**Figure 6.**  
Structures of U50,488, THC, WIN 55,212-2, and CP 55,940



**Figure 7.**  
Structures of UPHIT and DIPPA



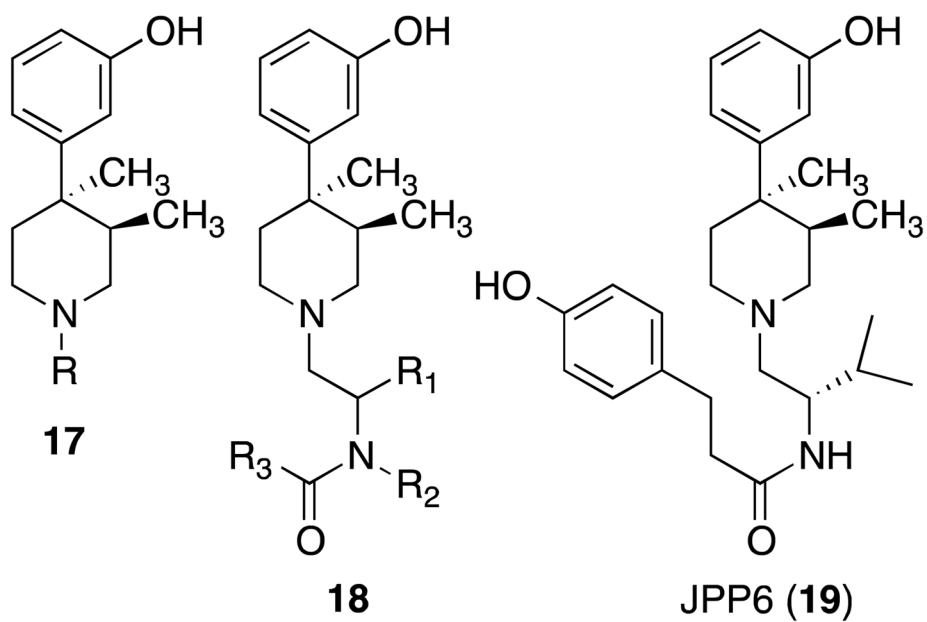
Tyr-D-Ala-Gly-N<sup>α</sup>-Me-Phe-Gly-ol

DAMGO (15)

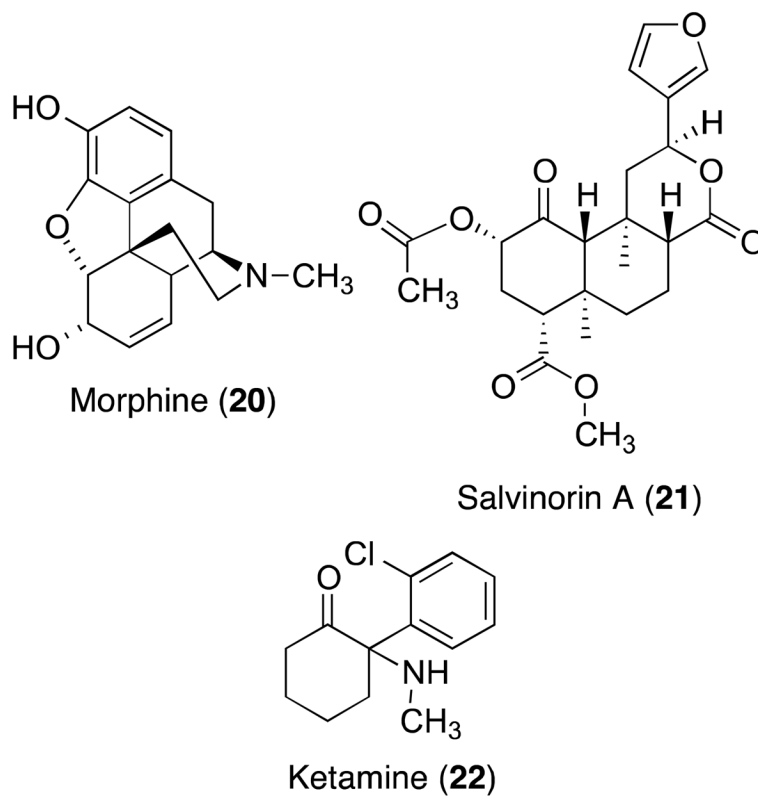
Tyr-c[D-Pen-Gly-Phe-D-Pen]-OH

DPDPE (16)

**Figure 8.**  
Structures of U69,593, bremazocine, DAMGO, and DPDPE

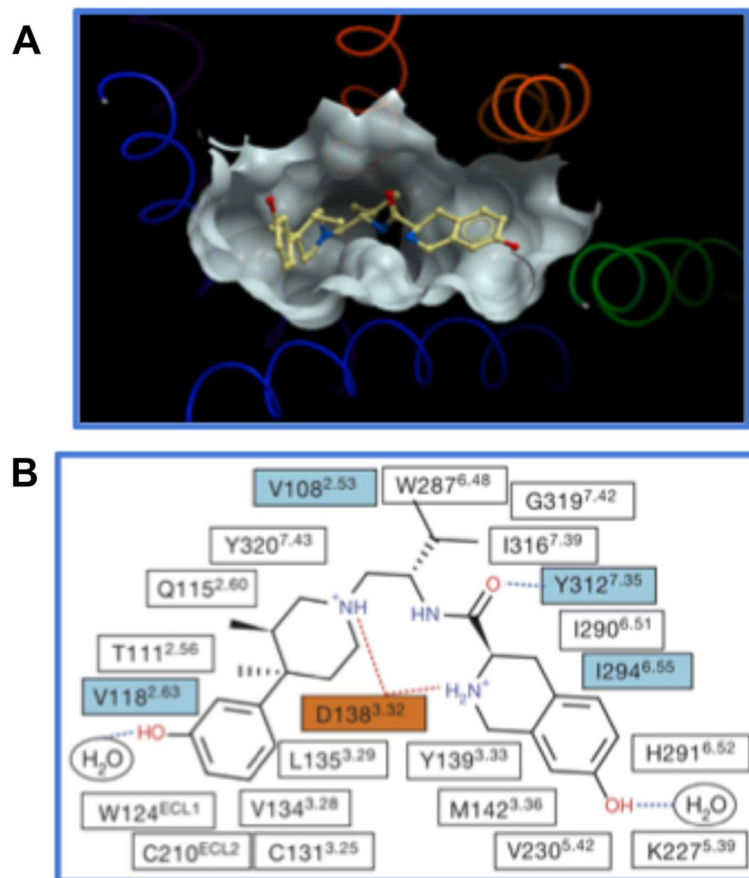


**Figure 9.**  
Structures of 17, 18, and 19



**Figure 10.**  
Structures of morphine, Salvinorin A, and ketamine





**Figure 11.**  
 Binding of JDtic in human KOR crystal structure  
 (A) JDtic in the binding pocket of the crystal structure.  
 (B) Diagram of JDtic interaction in the binding pocket side chain at 4.5 Å cut-off.  
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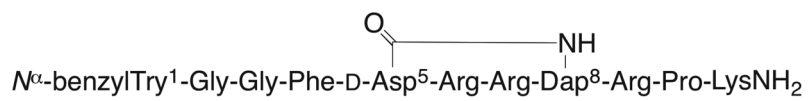
[*N,N*-diallyl,*D*-Pro<sup>10</sup>]Dyn A-(1-11) (**25**)

[*N,N*-diCPM,*D*-Pro<sup>10</sup>]Dyn A-(1-11) (**26**)

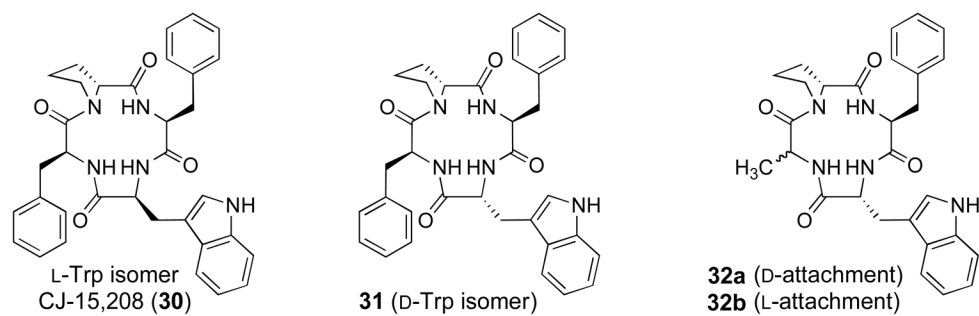
[Pro<sup>3</sup>]Dyn A-(1-11)NH<sub>2</sub> (**27**)

AcPhe-Phe-Phe-Arg-Leu-Arg-Arg-D-Ala-Arg-Pro-LysNH<sub>2</sub> (**28**, arodyn)

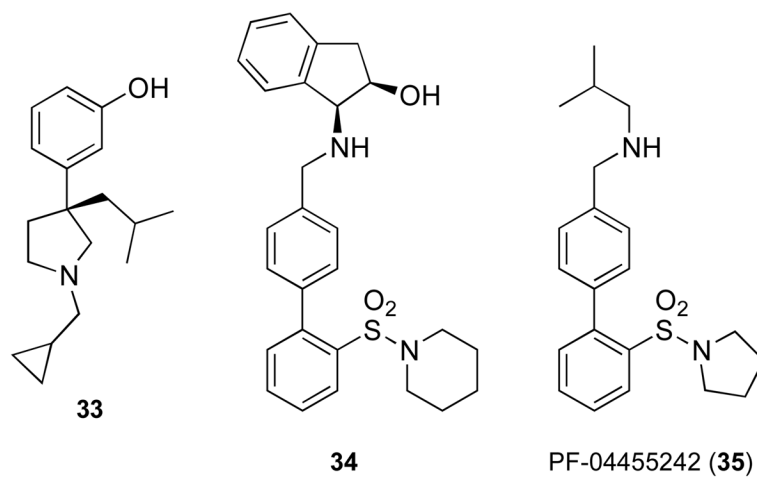
**Figure 12.**  
Structures of opioid peptides **25–28**



**Figure 13.**  
Structure of zyklophin (**29**)

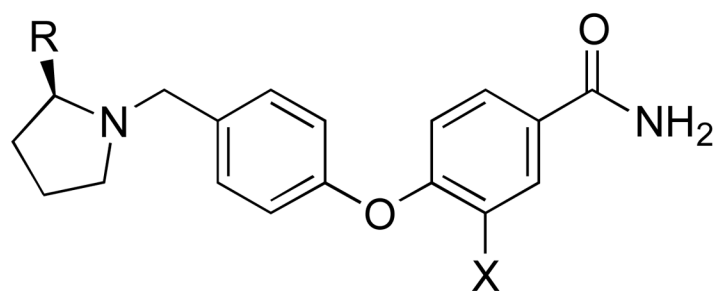


**Figure 14.**  
Structures of CJ-15,208 and analogues **31** and **32a–b**

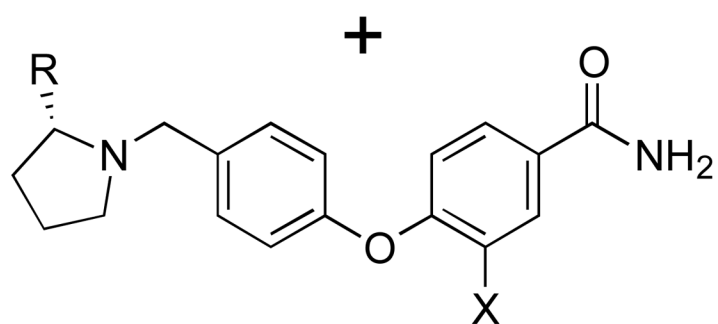


**Figure 15.**  
Structures of **33**, **34**, and PF-04455242 (**35**)

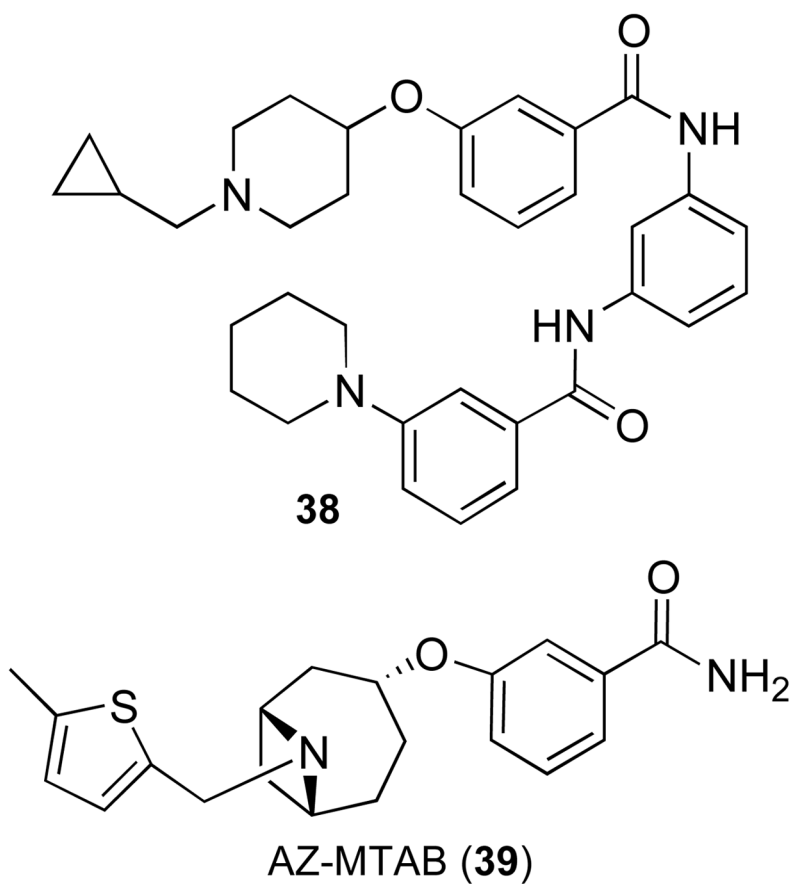




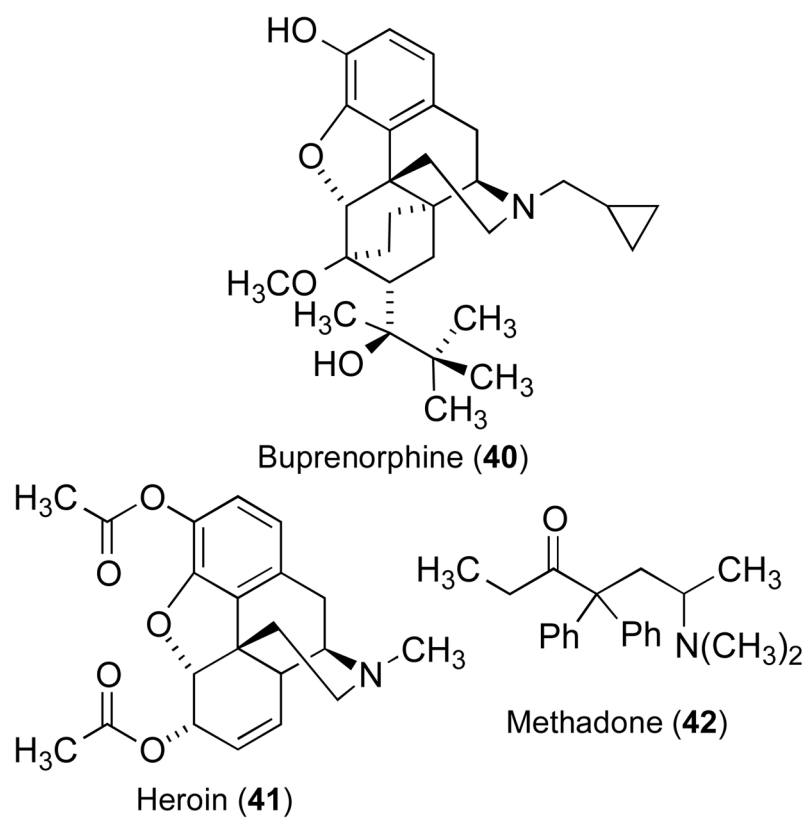
LY2456302

**36a**, (S) R = 3,5-dimethylphenyl, X = F**36b**, (S) R = 3-pyridyl, X = Cl**36c**, (S) R = 3-fluorophenyl, X = F**37a**, (S) R = 3,5-dimethylphenyl, X = F**37b**, (S) R = 3-pyridyl, X = Cl**37c**, (S) R = 3-fluorophenyl, X = F

**Figure 16.**  
Structures of aminobenzoyloxyarylamides **36a–c** and **37a–c**



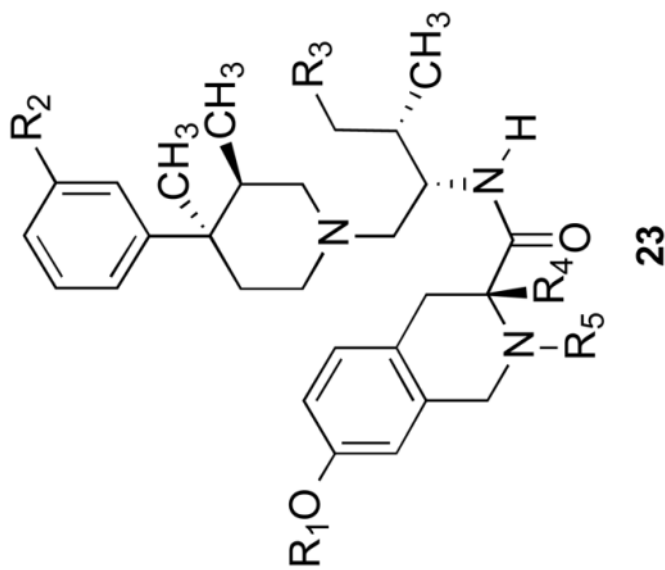
**Figure 17.**  
Structures of **38** and AZ-MTAB



**Figure 18.**  
Structures of buprenorphine, heroin, and methadone

Table 1

Inhibition of Agonist Stimulated [<sup>35</sup>S]GTP-γS Binding by Compounds in Cloned Human MOR, DOR, and KOR



Compd	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>	R <sub>5</sub>	K <sub>c</sub> (nM)			μ/κ	δ/κ
						DAMGO, μ	DPDPE, δ	U69,593, κ		
<b>2</b> (nor-BNI)						26	29	0.05	520	580
<b>3</b> (JDTic)	H	OH	H	H	H	3.41	79.3	0.01	341	7930
<b>23a</b>	CH <sub>3</sub>	OH	H	H	H	51	118	0.06	850	1966
<b>23b</b>	H	OCH <sub>3</sub>	H	H	H	24	21.2	0.037	649	573
<b>23c</b>	H	OH	CH <sub>3</sub>	H	H	3	24	0.03	100	800
<b>23d</b>	H	OH	H	CH <sub>3</sub>	H	3.6	854	0.03	120	28500
<b>23e</b>	H	OH	H	H	CH <sub>3</sub>	210	491	0.16	1313	3070
<b>23f</b>	H	CONH2	H	H	H	21	480	0.12	175	400

Table 2

Inhibition of Agonist Stimulated [<sup>35</sup>S]GTPγS Binding by Compounds in Cloned Human MOR, DOR, and KOR

Compd	K <sub>e</sub> (nM)				
	DAMGO, μ	DPDPE, δ	U69,593, κ	μ/κ	δ/κ
<b>2</b> (nor-BNI)	26	29	0.05	520	580
<b>3</b> (JDTic)	25	76	0.02	1250	3800
<b>24a</b> (KAA1)	42	33	0.24	175	138
<b>24b</b>	176	313	0.47	374	666
<b>24c</b>	259	77	2.2	118	35
<b>24d</b>	82	186	0.87	94	214
<b>24e</b>	48	13	0.09	533	144
<b>24f</b>	26	32	0.30	87	107
<b>24g</b>	52	62	0.09	578	689
<b>24h</b>	4.6	25	0.07	66	357
<b>24i</b>	28	25	0.04	700	625

