# Toward Directing Opioid Receptor Signaling to Refine Opioid Therapeutics

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Supplemental Information

### TABLE S1. Opioid Effects in Genetic Models with Disrupted MOR Regulation

### Antinociception and Tolerance

Genetic Model	Drug	Response	Effect Relative to WT	Ref
βarrestin2-KO mice	Morphine	Hot plate latency	Enhanced, prolonged	8,11,10, 23
	Morphine (sc daily, pellet)	Hot plate tolerance	Decreased	9,11
	Morphine (pump)	Hot plate tolerance	Decreased	23
	Morphine	Tail flick latency	Enhanced, prolonged	11,12
	Morphine (sc daily)	Tail flick tolerance	Delayed, but present	11
	Heroin	Hot plate latency	Enhanced, prolonged	10
	Etorphine	Hot plate latency	No change	10
	Fentanyl	Hot plate latency	No change	10, 23
	Fentanyl (pump)	Hot plate tolerance	No change	23
	Oxycodone	Hot plate latency	No change	23
	Oxycodone (pump)	Hot plate tolerance	No change	23
	Methadone	Hot plate latency	No change	23
	Methadone (pump)	Hot plate tolerance	No change	23
βarrestin2 Antigene mice	Morphine	Hot plate latency	Enhanced	13
	Morphine (sc daily)	Hot plate tolerance	Decreased	13
βarrestin2 siRNA PAG mice	Morphine	Hot plate latency	Enhanced	14
	Morphine (sc daily)	Hot plate tolerance	Decreased	14
βarrestin2 siRNA it rats	Morphine	Tail flick latency	Enhanced, prolonged	15
	Morphine (it daily)	Tail flick tolerance	Decreased	15
βarrestin1-KO mice	Morphine	Hot plate latency	No change	10
βarrestin1 siRNA PAG mice	Morphine	Hot plate latency	No change	14
	Morphine (sc daily)	Hot plate tolerance	No change	14
GRK3, 4, or 6-KO mice	Morphine	Hot plate latency	No change	18, 19, 24
GRK3-KO mice	Morphine (pellet)	Hot plate tolerance	No change	24
	Morphine (sc daily)	Hot plate tolerance	No change	19
	Fentanyl	Hot plate latency	No change	24
	Fentanyl (pump)	Hot plate tolerance	Decreased	24
	Fentanyl (sc daily)	Hot plate acute tolerance	Decreased	19
	Etonitazene (sc daily)	Hot plate tolerance	Decreased	19
GRK5-KO mice	Morphine	Hot plate latency	Decreased	19
	Morphine (sc daily)	Hot plate tolerance	No change	19
GRK6-KO mice	Morphine (pump)	Hot plate tolerance	No change	23
Phosphorylation deficient C	Morphine	Hot plate latency	Enhanced	21
terminus of MOR mice	Morphine (pump)	Hot plate tolerance	Decreased	21
	Fentanyl	Hot plate latency	Enhanced	21
	Fentanyl (pump)	Hot plate tolerance	Decreased	21
MOR S375A mice	Fentanyl (sc daily)	Hot plate tolerance	Decreased	25
	Etonitazine (sc daily)	Hot plate tolerance	Decreased	25
	Morphine (sc daily)	Hot plate tolerance	No change	25
MOR C-terminus truncation mice	Morphine (sc daily)	Tail flick tolerance	Decreased	22

#### Physical Dependence and Withdrawal

βarrestin2-KO mice	Morphine (pellet)	Naloxone-precipitated withdrawal	No change	9
	Morphine (pump)	Naloxone-precipitated withdrawal	Decreased	23
βarrestin2 siRNA i.t. rats	Morphine (it daily)	Naloxone-precipitated withdrawal	Decreased	15
GRK3-KO mice	Fentanyl (pump)	Naloxone-precipitated withdrawal	No change	24
GRK5-KO mice	Morphine (sc daily)	Naloxone-precipitated withdrawal	Decreased	19
GRK6-KO mice	Morphine (pump)	Naloxone-precipitated withdrawal	No change	20
S375A mice	Morphine (pump)	Naloxone-precipitated withdrawal	No change	21
	Fentanyl (pump)	Naloxone-precipitated withdrawal	No change	21
Phosphorylation deficient C	Morphine (pump)	Naloxone-precipitated withdrawal	No change	21
terminus of MOR mice	Fentanyl (pump)	Naloxone-precipitated withdrawal	No change	21
MOR C-terminus truncation	Morphine (sc daily)	Naloxone-precipitated withdrawal	Decreased	22
mice				
exon4-encoded C terminus	Morphine (sc daily)	Naloxone-precipitated withdrawal	Decreased	22
deletion mice exon7-encoded C terminus	Morphine (sc daily)	Naloxone-precipitated withdrawal	No change	22
deletion mice	worphine (So daily)	Nalozone-precipitated withdrawar	No change	22

Barrestin2-KO mice         Morphine         Accumulation of fecal boli         Increased         33           GRK6-KO mice         Morphine         Accumulation transit - orally administered charcoal meal Large intestine transit - colonic bead expulsion         Decreased delay         33           GRK6-KO mice         Morphine         Accumulation of fecal boli         Increased         20           MOR S375A mice         Morphine         Accumulation of fecal boli         Increased delay         20           MOR S375A mice         Morphine         Accumulation of fecal boli         No change         21           Phosphorylation deficient C         Morphine         Accumulation of fecal boli         No change         21           MOR C-terminus fruncation         Morphine         Samall intestinal transit - orally administered charcoal meal scont-encoded C terminus         Morphine         Small intestinal transit - orally administered charcoal meal mice         No change         21           Respiratory Suppression         Fentanyl         Accumulation of fecal boli         No change         22           MOR S375A mice         Morphine         Small intestinal transit - orally administered charcoal meal meal starset transit - orally         No change         21           MOR C-terminus function         Morphine         Respiratory suppression         No change         21	Constipation				
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Bead expulsion         Accumulation of fecal boli         Increased         20           MOR S375A mice         Morphine         Accumulation of fecal boli         No change         20           MOR S375A mice         Morphine         Accumulation of fecal boli         No change         21           Phosphorylation deficient C         Morphine         Accumulation of fecal boli         No change         21           MOR Career         Fentanyl         Accumulation of fecal boli         No change         21           MOR Career         Morphine         Accumulation of fecal boli         No change         21           MOR Career         Morphine         Small intestinal transit - orally administered charcoal meal         No change         22           MOR Career         Morphine         Small intestinal transit - orally administered charcoal meal         No change         22           Respiratory Suppression         Morphine         Small intestinal transit - orally administered charcoal meal         No change         21           Phosphorytation deficient C         Morphine         Small intestinal transit - orally administered charcoal meal         No change         21           Respiratory Suppression         Morphine         Small intestinal transit - orally administered charcoal meal         Small intestinal transit - orally administered charcoal meal				No change	33
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Fentanyl CPP No change 21	terminus of MOR mice	Fentanyl	Locomotor activity		
,		Morphine		No change	
		Fentanyl		No change	21

Subcutaneous, sc; intrathecal, it; conditioned-place preference, CPP.

#### TABLE S2. In Vitro Pharmacology of Compounds Compared in this Review for Biased Agonism.

	MOR Receptor Activity				
Compound	Assay 1 G protein	Assay 2 βarrestin2	<b>Bias Calculation Method</b>	Conclusion	Ref
		βarrestin2 enzyme complementation	Black-Leff operational model-		
DAMGO	GTP $\gamma$ S binding, cAMP accumulation	assay and BRET	no data provided*	Equal between assays	46
		βarrestin2 enzyme complementation			
	GTP $\gamma$ S binding, cAMP accumulation	assay	Black-Leff operational model	Equal between assays	60
Morphine	CTDvS binding oAMD occumulation	βarrestin2 enzyme complementation	Black-Leff operational model-	Equal between energy	45
Morphine	GTP <sub>γ</sub> S binding, cAMP accumulation	assay and BRET βarrestin2 enzyme complementation	no data provided*	Equal between assays	40
	GTP $\gamma$ S binding, cAMP accumulation	assay	Black-Leff operational model	Equal between assays	60
	Stri yo binding, crawin accomutation	βarrestin2-GFP translocation &	Black-Len operational model	ERK1/2 activation with poor βarrestin2	00
Herkinorin	ERK1/2 phosphorylation	internalization by confocal microscopy	None applied	recruitment & no internalization	43
	p			G protein with no βarrestin2	
		βarrestin2-GFP translocation +/- GRK2		translocation to the plasma membrane	
	$GTP\gamma S$ binding	by confocal microscopy	None applied	even when GRK2 is overexpressed	45
			Black-Leff operational model-	Modest G protein bias with βarrestin2	
	cAMP accumulation +GRK2	βarrestin2 BRET +GRK2	no data provided*	recruitment	46
		βarrestin2 enzyme complementation	<b>F</b> . 1. <b>P</b>		40
Oliceridine	cAMP accumulation	assay βarrestin2 enzyme complementation	Equiactive comparison model Black-Leff operational model-	G protein bias G protein with undetectable βarrestin2	48
	GTP $\gamma$ S binding, cAMP accumulation	assay and BRET	no data provided*	enzyme complementation or BRET	46
	GTT yo binding, cAim accumulation		Black-Leff operational model-	enzyme complementation of BRE1	40
	cAMP accumulation +GRK2	ßarrestin2 BRET +GRK2	no data provided*	G protein bias	46
		βarrestin2 enzyme complementation	Black-Leff operational model-	G protein with undetectable βarrestin2	
PZM21	GTP $\gamma$ S binding, cAMP accumulation	assay and BRET	no data provided*	enzyme complementation or BRET	46
			Black-Leff operational model-		
	cAMP accumulation +GRK2	βarrestin2 BRET +GRK2	no data provided*	G protein bias	46
		βarrestin2 enzyme complementation			
Mitragynine	$GTP\gamma S$ binding	assay	None applied	G protein bias	58
SR-14968	CTD <sub>2</sub> S hinding oAMD accumulation	βarrestin2 enzyme complementation	Diack Loff anarational model	C protoin high	60
SK-14908	GTP $\gamma$ S binding, cAMP accumulation	assay βarrestin2 enzyme complementation	Black-Leff operational model	G protein bias	60
SR-17018	GTP $\gamma$ S binding, cAMP accumulation	assay	Black-Leff operational model	G protein bias	60
		ussuy			00

\*The methods state using the Black and Leff Operational model to compare bias, but no values or calculations are provided in the text. In cases where efficacy of the response is low, the manuscript (46) states that the data were not fit to the model.

GTP $\gamma$ S binding refers to 35S-GTP $\gamma$ S binding in membranes.

βarrestin2 enzyme complementation assay (EFC) is the DiscoveRx PathHunter® assay. BRET is a Bioluminescence Resonance Energy Transfer assay

# TABLE S3. In Vivo Responses to Biased Agonists.

#### Antinociception and Tolerance

Herkinorin	Subject				
	Rats	Inflammation pain	Acute flinch response in the paw formalin test Tolerance flinch response in the paw formalin test	Enhanced potency but localized antinociception Decreased effect	44 44
Oliceridine	Mice	Central Antinociception Spinal reflex	Hot plate latency Tail flick latency	Enhanced potency with faster onset for antinociception Enhance potency	48 49
		Antinociception	Tail flick tolerance	Decreased effect	49, 51
		Nociceptive sensitization	Opioid-induced hyperalgesia (OIH)	Decreased effect	51
	Rats	Central Antinociception	Hot plate latency	Enhance potency	48
	T Cato	Contrait / Intinocicoption	Hot plate latency	Similar effect (compared to oxycodone)	50
		Spinal Antinociception	Tail flick latency	Enhance potency	48
		Post-operative pain	Hindpaw incisional model	Enhance potency	48
	Human clinical trials	Opioid analgesia	Cold-pain test	Enhanced analgesia with fast onset and similar duration	52
	chinical trials	Post-operative pain	Average pain scores	Similar effect	54
			Treatment responders using categorical and numeric rating scale (NRS) and drug demand using patient-controlled-analgesia (PCA) over 48 hours	Similar analgesic effect with fast onset and similar duration	56
			Treatment responders using categorical and numeric rating scale (NRS) and drug demand using patient-controlled-analgesia (PCA) over 24 hours	Similar analgesic effect with fast onset	55
PZM21	Mice	Central Antinociception	Hot plate latency (affective)*	Decreased at equal dose	46
			Hot plate latency	Similar	57
			Hot plate tolerance	Similar	57
		Spinal reflex Antinociception	Tail flick latency	No Response	46
		Inflammation pain	Acute lick response in the paw formalin test	Similar	46
Mitragynine and derivatives	Mice	Central Antinociception	Hot plate latency	Similar effect	58
		Spinal reflex Antinociception	Tail flick latency	Mitragynine showed decreased potency while its derivatives displayed enhanced potency with shorter duration.	58
		Antinociception Tolerance	Not specified	Mitragynine derivative showed slower development of tolerance	58
SR-14968	Mice	Central Antinociception	Hot plate latency	Enhanced potency	60
		Spinal reflex Antinociception	Tail flick latency	Enhanced potency	60
	Rats	Spinal reflex Antinociception	Tail flick latency	Enhanced potency	63
SR-17018	Mice	Central Antinociception	Hot plate latency	Similar effect	60
		•	Hot plate tolerance	Decreased effect	62

Oliceridine	Mice	Constipation	Accumulation of fecal boli	Decreased constipation	48
			Accumulation of fecal boli	Similar	49

			Small intestinal transit - orally administered charcoal meal	Less transit delay	48
			Large intestine transit - colonic bead	Less transit delay	48
			expulsion Large intestine transit - colonic bead	Similar	49
		Physical dependence and	expulsion Naloxone-precipitated withdrawal	Similar effect but to a lesser extent	51
		withdrawal			
	Rats	Respiratory function	Respiratory suppression	Decreased effect	48
		Abuse liability	Intracranial self-stimulation	Similar effect	49
			Self-administration	Similar effect (compared to oxycodone instead of	50
	11			morphine)	50
	Human clinical trials	Respiratory function	Magnitude, duration, and frequency of depressed ventilatory response to hypercapnia	Similar effect but transient	52
			Hypoventilation and respiratory suppression	Decreased effect	54
			Respiratory safety burden (RSB) using zero-	Decreased effect	56
			inflated gamma mixture model		
			Respiratory safety burden (RSB) using zero-	Similar effect	55
			inflated gamma mixture model		
		Gastrointestinal adverse events (AEs)	Drug Effects Questionnaire (DEQ)	Decreased and less severe effect	52
			Upper gastrointestinal effects	Decreased and less severe effect	54
			Gastrointestinal adverse effects (AEs)	Dose-related AEs but less frequent and less severe	56
			Gastrointestinal adverse effects (AEs)	Dose-dependent AEs but less frequent	55
		Abuse liability	Drug Effects Questionnaire (DEQ)	Similar but with greater therapeutic index	52
	Human clinical trials	Respiratory function	Magnitude, duration, and frequency of depressed ventilatory response to hypercapnia	Similar effect but transient	52
			Hypoventilation and respiratory suppression	Decreased effect	54
			Respiratory safety burden (RSB) using zero-	Decreased effect	56
			inflated gamma mixture model		
PZM21	Mice	Respiratory suppression	Whole-body plethysmography	Decreased effect	46
			Whole-body plethysmography	Similar effect	57
		Respiratory suppression tolerance	Whole-body plethysmography	Not acquired (similar to morphine in this study)	57
		Reward	Conditioned-place preference (CPP)	No CPP	46
		Opioid-induced	Open-field locomotor activity	Decreased effect	46
		psychomotor effect			
Mitragynine	Mice	Constipation	Small intestinal transit - orally administered	Decreased effect	58
and			charcoal meal		
derivatives					
		Respiratory suppression	%O2 pulse oximeter system	Decreased effect	58
		Reward	Conditioned-place preference (CPP) or aversion (CPA)	Unclear CPP or CPA	58
SR-14968	Mice	Respiratory suppression	%O2 pulse oximeter system	Decreased effect	60
	Rats	Abuse-liability	Discriminative stimulus effects	Similar effect but with enhanced potency and efficacy ratios to produce antinociception over abuse-related discriminative stimulus effects	63
SR-17018	Mice	Respiratory suppression	%O2 pulse oximeter system	Decreased effect	60
51-17010	MICE	Dependence and	Assessment of somatic signs from SR-17018	Faster recovery	62
*P7M21 affective	response to hot	withdrawal	withdrawal		

\*PZM21 affective response to hot plate assay.