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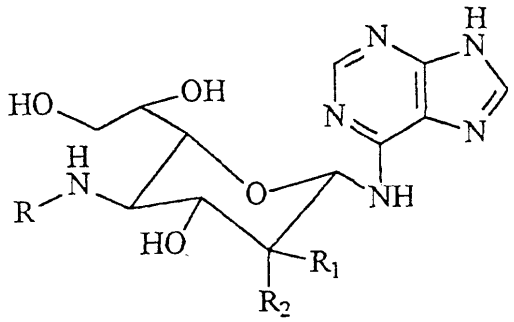
(54)【発明の名称】スピカマイシン誘導体を用いての疼痛の低減または予防法

(57)【要約】
水溶性スピカマイシン誘導体を投与することにより、疼痛軽減を提供する方法。疼痛仲介剤の使用法も提供される。

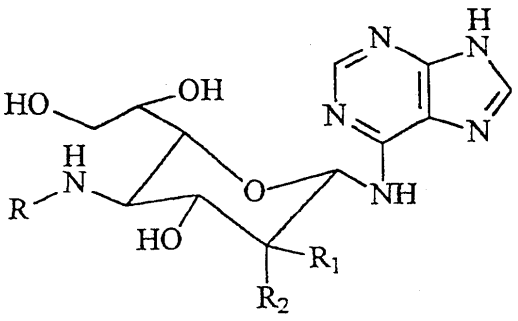
【特許請求の範囲】
【請求項１】
疼痛軽減を提供する方法であって、
疼痛軽減を必要とする被験者を同定する段階；および
被験者における有意な疼痛軽減を提供するのに有効な量の式ＩＩの化合物またはその塩を被験者に投与する段階
を含む方法：
【化１】



式 II

（式中、R_1およびR_2は互いに異なり、－Ｈまたは－ＯＨを表し、かつＲは（１）一つもしくは二つの炭素原子を有する置換もしくは無置換アルキル、または（２）－Ｈを表す）。
【請求項２】
Ｒが置換アルキルである、請求項１に記載の方法。
【請求項３】
Ｒが二つの炭素原子を有する、請求項１に記載の方法。
【請求項４】
Ｒがペプチド結合を含む、請求項１に記載の方法。
【請求項５】
Ｒがアミノ基を含む、請求項１に記載の方法。
【請求項６】
アミノ基が一級アミノ基である、請求項５に記載の方法。
【請求項７】
Ｒが－ＣＯＣＨ$_2$ＮＨ$_2$である、請求項１に記載の方法。
【請求項８】
R_1が－Ｈであり、かつR_2が－ＯＨである、請求項７に記載の方法。
【請求項９】
R_1が－Ｈであり、かつR_2が－ＯＨである、請求項１に記載の方法。
【請求項１０】
疼痛が神経障害性疼痛である、請求項１に記載の方法。
【請求項１１】
疼痛がヘルペス後神経痛、幻肢もしくは切断端痛、糖尿病性神経障害、後天性免疫不全症候群神経障害、背痛、内臓痛、または慢性膵炎性神経障害である、請求項１０に記載の方法。
【請求項１２】
疼痛がオピオイド抵抗性である、請求項１に記載の方法。
【請求項１３】

被験者が哺乳類である、請求項１に記載の方法。
【請求項１４】
被験者がヒトである、請求項１に記載の方法。
【請求項１５】
化合物が全身投与される、請求項１に記載の方法。
【請求項１６】
化合物が被験者の疼痛部位に投与される、請求項１に記載の方法。
【請求項１７】
化合物がインプラントを介して投与される、請求項１に記載の方法。
【請求項１８】
インプラントが化合物の遅延放出を提供する、請求項１７に記載の方法。
【請求項１９】
化合物が静脈内投与される、請求項１に記載の方法。
【請求項２０】
投与量が体表面積１m^2あたり約１ｎｇから４ｍｇである、請求項１に記載の方法。
【請求項２１】
投与量が患者体表面積１m^2あたり約８０ｎｇから１ｍｇである、請求項１に記載の方法。
【請求項２２】
投与量が体重１ｋｇあたり約１０ｍｇから１００ｍｇである、請求項１に記載の方法。
【請求項２３】
投与量が体重１ｋｇあたり約１００ｍｇである、請求項２２に記載の方法。
【請求項２４】
化合物が水溶液で投与される、請求項１に記載の方法。
【請求項２５】
侵害受容性疼痛よりも神経障害性疼痛が選択的に軽減される、請求項１に記載の方法。
【請求項２６】
神経障害性疼痛治療のための標的として、遺伝子またはその遺伝子によってコードされる被験ポリペプチドを査定する方法であって、
式ＩＩの化合物またはその塩を被験ポリペプチドと接触させる段階；および
式ＩＩの化合物またはその塩と被験ポリペプチドとの結合親和性を測定する段階を含む方法：
【化２】



式 II

（式中：
各Ｒ基は独立にＨ、または１個から３個の独立のＲ^{3}もしくはＲ^{4}で置換された１個から５個の炭素原子を有するアルキル基であってもよく；
各Ｒ^{3}は独立に、いずれも１個～３個の独立のＲ^{5}で選択的に置換されたヘテロシクリルまたはヘテロアリールであり；
各Ｒ^{4}は独立にハロゲン、酸素、硫黄、ＣＦ$_3$、ＳＲ^6、ＯＲ^6、ＯＣ（Ｏ）Ｒ^6、ＮＲ

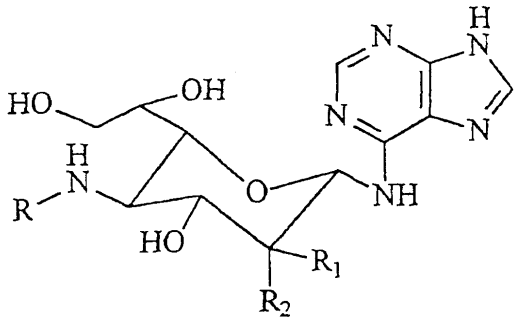
^{6} R^{6}、NR^{6} R^{7}、COOR^{6}、C（O）R^{6}、またはC（O）NR^{6} R^{6} であり；
各R^{5} は独立にC１～C１０アルキル；ハロ；ハロアルキル；SR^{6}；OR^{6}；NR^{6} R^{6}；COOR^{6}；NO_2；CN；C（O）R^{6}；C（O）NR^{6} R^{6}；OC（O）R^{6}；S（O）$_2$ R^{6}；S（O）$_2$ NR^{6} R^{6}；NR^{6} C（O）NR^{6} R^{6}；NR^{6} C（O）R^{6}；NR^{6}（COOR^{6}）；NR^{6} C（O）R^{8}；NR^{6} S（O）$_2$ NR^{6} R^{6}；NR^{6} S（O）$_2$ R^{6}；NR^{6} S（O）$_2$ R^{8}；またはR^{4} もしくはR^{8} で置換されたC１～C１０アルキルであり；
各R^{6} は独立にH、C１～C１０アルキル；C２～C１０アルケニル；C２～C１０アルキニル；C３～C１０シクロアルキル；R^{8}；またはR^{8} で置換されたC１～C１０アルキルであり；
各R^{7} は独立にCOOR^{9}、C（O）NR^{9} R^{9}、S（O）$_2$ R^{9}；またはS（O）$_2$ NR^{9} R^{9} であり；
各R^{8} は独立にアリール、ヘテロアリール、またはヘテロシクリルであり；かつ　各R^{9} は独立にH、C１～C１０アルキル、アリール、ヘテロアリール、またはヘテロシクリルである）。

【請求項２７】
R_1 およびR_2 は互いに異なり、－Hまたは－OHを表し、かつRは（１）一つもしくは二つの炭素原子を有する置換もしくは無置換アルキル、または（２）－Hを表す、請求項２６に記載の方法。

【請求項２８】
各Rは独立にH、または１個から３個の独立のR^{3} もしくはR^{4} で置換された１から２個の炭素原子を有するアルキル基であってもよい、請求項２６に記載の方法。

【請求項２９】
疼痛軽減を提供する方法であって、
疼痛軽減を必要とする被験者を同定する段階；および
被験者における有意な疼痛軽減を提供するのに有効な量の式ＩＩの化合物またはその塩を被験ポリペプチドと共に被験者に投与する段階
を含む方法：

【化３】



式 II

（式中
各R基は独立にH、または１個から３個の独立のR^{3} もしくはR^{4} で置換された１個から５個の炭素原子を有するアルキル基であってもよく；
各R^{3} は独立に、いずれも１個～３個の独立のR^{5} で選択的に置換されたヘテロシクリルまたはヘテロアリールであり；
各R^{4} は独立にハロゲン、酸素、硫黄、CF_3、SR^{6}、OR^{6}、OC（O）R^{6}、NR^{6} R^{6}、NR^{6} R^{7}、COOR^{6}、C（O）R^{6}、またはC（O）NR^{6} R^{6} であり；
各R^{5} は独立にC１～C１０アルキル；ハロ；ハロアルキル；SR^{6}；OR^{6}；NR^{6} R^{6}；COOR^{6}；NO_2；CN；C（O）R^{6}；C（O）NR^{6} R^{6}；OC（O）R^{6}；

Ｓ（Ｏ）$_2$Ｒ^6；Ｓ（Ｏ）$_2$ＮＲ^{6}Ｒ^6；ＮＲ^{6}Ｃ（Ｏ）ＮＲ^{6}Ｒ^6；ＮＲ^{6}Ｃ（Ｏ）Ｒ^6；ＮＲ^6（ＣＯＯＲ^6）；ＮＲ^{6}Ｃ（Ｏ）Ｒ^8；ＮＲ^{6}Ｓ（Ｏ）$_2$ＮＲ^{6}Ｒ^6；ＮＲ^{6}Ｓ（Ｏ）$_2$Ｒ^6；ＮＲ^{6}Ｓ（Ｏ）$_2$Ｒ^8；またはＲ^{4}もしくはＲ^{8}で置換されたＣ１～Ｃ１０アルキルであり；
各Ｒ^{6}は独立にＨ、Ｃ１～Ｃ１０アルキル；Ｃ２～Ｃ１０アルケニル；Ｃ２～Ｃ１０アルキニル；Ｃ３～Ｃ１０シクロアルキル；Ｒ^8；またはＲ^{8}で置換されたＣ１～Ｃ１０アルキルであり；
各Ｒ^{7}は独立にＣＯＯＲ^9、Ｃ（Ｏ）ＮＲ^{9}Ｒ^9、Ｓ（Ｏ）$_2$Ｒ^9；またはＳ（Ｏ）$_2$ＮＲ^{9}Ｒ^{9}であり；
各Ｒ^{8}は独立にアリール、ヘテロアリール、またはヘテロシクリルであり；かつ　各Ｒ^{9}は独立にＨ、Ｃ１～Ｃ１０アルキル、アリール、ヘテロアリール、またはヘテロシクリルである）。

【請求項３０】
疼痛が神経障害性疼痛である、請求項２９に記載の方法。

【請求項３１】
疼痛がヘルペス後神経痛、幻肢もしくは切断端痛、糖尿病性神経障害、後天性免疫不全症候群神経障害、背痛、内臓痛、または慢性膵炎性神経障害である、請求項３０に記載の方法。

【請求項３２】
疼痛がオピオイド抵抗性である、請求項２９に記載の方法。

【請求項３３】
被験者が哺乳類である、請求項２９に記載の方法。

【請求項３４】
被験者がヒトである、請求項２９に記載の方法。

【請求項３５】
化合物が全身投与される、請求項２９に記載の方法。

【請求項３６】
化合物が被験者の疼痛部位に投与される、請求項２９に記載の方法。

【請求項３７】
化合物がインプラントを介して投与される、請求項２９に記載の方法。

【請求項３８】
インプラントが化合物の遅延放出を提供する、請求項３７に記載の方法。

【請求項３９】
化合物が静脈内投与される、請求項２９に記載の方法。

【請求項４０】
投与量が体表面積１ｍ^{2}あたり約１ｎｇから４ｍｇである、請求項２９に記載の方法。

【請求項４１】
投与量が患者体表面積１ｍ^{2}あたり約８０ｎｇから１ｍｇである、請求項２９に記載の方法。

【請求項４２】
投与量が体重１ｋｇあたり約１０ｍｇから１００ｍｇである、請求項２９に記載の方法。

【請求項４３】
投与量が体重１ｋｇあたり約１００ｍｇである、請求項４２に記載の方法。

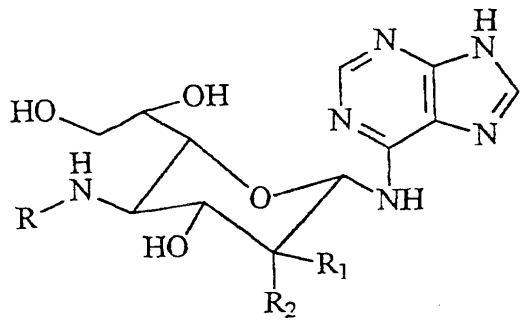
【請求項４４】
化合物が水溶液で投与される、請求項２９に記載の方法。

【請求項４５】
侵害受容性疼痛よりも神経障害性疼痛が選択的に軽減される、請求項２９に記載の方法。

【請求項４６】
疼痛仲介に関与する新規の遺伝子、受容体、またはペプチドを同定する方法であって、式ＩＩの化合物またはその塩を組織に投与する段階、組織を採取する段階、および公知また

は新規の遺伝子、受容体、またはペプチドの効果について組織を評価する段階を含む方法：

【化４】

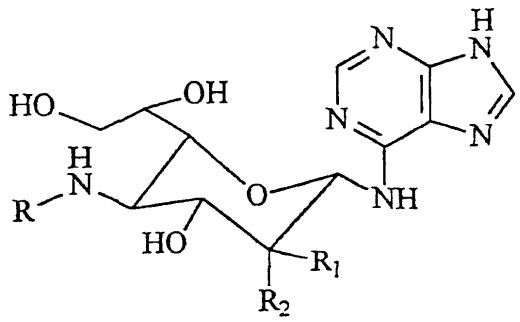


式 II

（式中
各Ｒ基は独立にＨ、または１から３個の独立のＲ[3]もしくはＲ[4]で置換された１から５個の炭素原子を有するアルキル基であってもよく；
各Ｒ[3]は独立に、いずれも１～３個の独立のＲ[5]で選択的に置換されたヘテロシクリルまたはヘテロアリールであり；
各Ｒ[4]は独立にハロゲン、酸素、硫黄、ＣＦ$_3$、ＳＲ[6]、ＯＲ[6]、ＯＣ（Ｏ）Ｒ[6]、ＮＲ[6]Ｒ[6]、ＮＲ[6]Ｒ[7]、ＣＯＯＲ[6]、Ｃ（Ｏ）Ｒ[6]、またはＣ（Ｏ）ＮＲ[6]Ｒ[6]であり；
各Ｒ[5]は独立にＣ１～Ｃ１０アルキル；ハロ；ハロアルキル；ＳＲ[6]；ＯＲ[6]；ＮＲ[6]Ｒ[6]；ＣＯＯＲ[6]；ＮＯ$_2$；ＣＮ；Ｃ（Ｏ）Ｒ[6]；Ｃ（Ｏ）ＮＲ[6]Ｒ[6]；ＯＣ（Ｏ）Ｒ[6]；Ｓ（Ｏ）$_2$Ｒ[6]；Ｓ（Ｏ）$_2$ＮＲ[6]Ｒ[6]；ＮＲ[6]Ｃ（Ｏ）ＮＲ[6]Ｒ[6]；ＮＲ[6]Ｃ（Ｏ）Ｒ[6]；ＮＲ[6]（ＣＯＯＲ[6]）；ＮＲ[6]Ｃ（Ｏ）Ｒ[8]；ＮＲ[6]Ｓ（Ｏ）$_2$ＮＲ[6]Ｒ[6]；ＮＲ[6]Ｓ（Ｏ）$_2$Ｒ[6]；ＮＲ[6]Ｓ（Ｏ）$_2$Ｒ[8]；またはＲ[4]もしくはＲ[8]で置換されたＣ１～Ｃ１０アルキルであり；
各Ｒ[6]は独立にＨ、Ｃ１～Ｃ１０アルキル；Ｃ２～Ｃ１０アルケニル；Ｃ２～Ｃ１０アルキニル；Ｃ３～Ｃ１０シクロアルキル；Ｒ[8]；またはＲ[8]で置換されたＣ１～Ｃ１０アルキルであり；
各Ｒ[7]は独立にＣＯＯＲ[9]、Ｃ（Ｏ）ＮＲ[9]Ｒ[9]、Ｓ（Ｏ）$_2$Ｒ[9]；またはＳ（Ｏ）$_2$ＮＲ[9]Ｒ[9]であり；
各Ｒ[8]は独立にアリール、ヘテロアリール、またはヘテロシクリルであり；かつ　各Ｒ[9]は独立にＨ、Ｃ１～Ｃ１０アルキル、アリール、ヘテロアリール、またはヘテロシクリルである）。

【請求項４７】
潜在的な疼痛薬の効力を査定する方法であって、被験化合物および式ＩＩの化合物を、被験化合物および式ＩＩの化合物の有効性の尺度または査定を提供する被験者または媒体に供することにより、式ＩＩの化合物またはその塩に対して被験化合物を評価する段階を含む方法：

【化５】



式 II

（式中
各Ｒ基は独立にＨ、または１から３個の独立のＲ[3]もしくはＲ[4]で置換された１から５個の炭素原子を有するアルキル基であってもよく；
各Ｒ[3]は独立に、いずれも１～３個の独立のＲ[5]で選択的に置換されたヘテロシクリルまたはヘテロアリールであり；
各Ｒ[4]は独立にハロゲン、酸素、硫黄、ＣＦ$_3$、ＳＲ[6]、ＯＲ[6]、ＯＣ（Ｏ）Ｒ[6]、ＮＲ[6]Ｒ[6]、ＮＲ[6]Ｒ[7]、ＣＯＯＲ[6]、Ｃ（Ｏ）Ｒ[6]、またはＣ（Ｏ）ＮＲ[6]Ｒ[6]であり；
各Ｒ[5]は独立にＣ１～Ｃ１０アルキル；ハロ；ハロアルキル；ＳＲ[6]；ＯＲ[6]；ＮＲ[6]Ｒ[6]；ＣＯＯＲ[6]；ＮＯ$_2$；ＣＮ；Ｃ（Ｏ）Ｒ[6]；Ｃ（Ｏ）ＮＲ[6]Ｒ[6]；ＯＣ（Ｏ）Ｒ[6]；Ｓ（Ｏ）$_2$Ｒ[6]；Ｓ（Ｏ）$_2$ＮＲ[6]Ｒ[6]；ＮＲ[6]Ｃ（Ｏ）ＮＲ[6]Ｒ[6]；ＮＲ[6]Ｃ（Ｏ）Ｒ[6]；ＮＲ[6]（ＣＯＯＲ[6]）；ＮＲ[6]Ｃ（Ｏ）Ｒ[8]；ＮＲ[6]Ｓ（Ｏ）$_2$ＮＲ[6]Ｒ[6]；ＮＲ[6]Ｓ（Ｏ）$_2$Ｒ[6]；ＮＲ[6]Ｓ（Ｏ）$_2$Ｒ[8]；またはＲ[4]もしくはＲ[8]で置換されたＣ１～Ｃ１０アルキルであり；
各Ｒ[6]は独立にＨ、Ｃ１～Ｃ１０アルキル；Ｃ２～Ｃ１０アルケニル；Ｃ２～Ｃ１０アルキニル；Ｃ３～Ｃ１０シクロアルキル；Ｒ[8]；またはＲ[8]で置換されたＣ１～Ｃ１０アルキルであり；
各Ｒ[7]は独立にＣＯＯＲ[9]、Ｃ（Ｏ）ＮＲ[9]Ｒ[9]、Ｓ（Ｏ）$_2$Ｒ[9]；またはＳ（Ｏ）$_2$ＮＲ[9]Ｒ[9]であり；
各Ｒ[8]は独立にアリール、ヘテロアリール、またはヘテロシクリルであり；かつ　各Ｒ[9]は独立にＨ、Ｃ１～Ｃ１０アルキル、アリール、ヘテロアリール、またはヘテロシクリルである）。

【発明の詳細な説明】

【０００１】

関連出願への相互参照

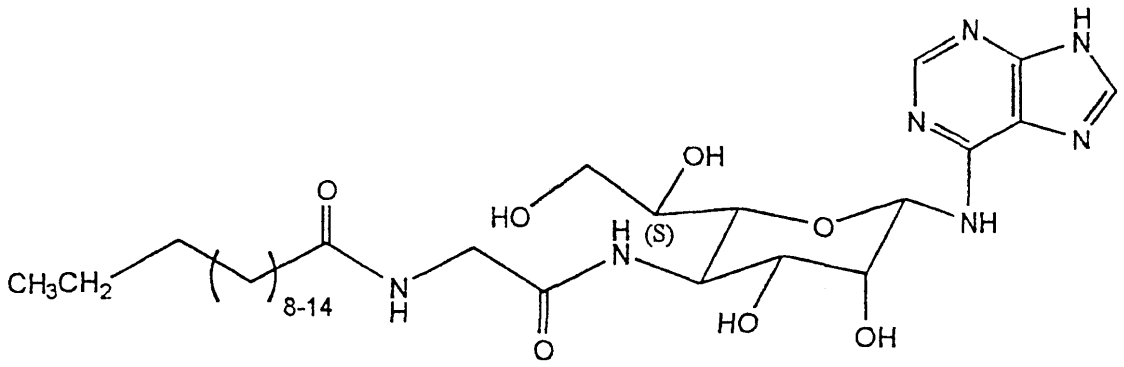
本出願は、２０００年９月２０日出願の米国特許仮出願第６０／２３４，３８２号の恩典を主張する。

【０００２】

発明の背景

スピカマイシン（ＳＰＭ）は、細菌ストレプトマイセス・アラノシニカス（Ｓｔｒｅｐｔｏｍｙｃｅｓ　ａｌａｎｏｓｉｎｉｃｕｓ）８７９－ＭＴ$_3$（ハヤカワ（Ｈａｙａｋａｗａ）ら、Ａｇｒｉｃ．Ｂｉｏｌ．Ｃｈｅｍ．４９：２６８５～２６９１、１９８５）によって産生される抗腫瘍抗生物質である。スピカマイシンおよびその誘導体は、疼痛軽減のためにも用いられる（米国特許第５，９０５，０６９号）。天然ＳＰＭは、脂肪酸部分においてのみ変動する下記の一般構造を有している：

【化６】



式Ｉ：スピカマイシン

スピカマイシンの合成変種およびそれらの抗腫瘍物質としての使用は、オタケ（Ｏｔａｋｅ）ら、米国特許第５，４６１，０３６号および第５，６３１，２３８号に記載されている。

【０００３】

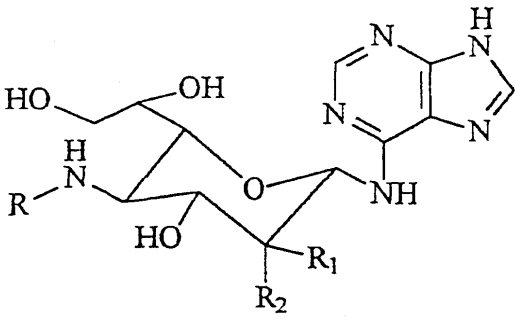
発明の概要

本発明は、スピカマイシン誘導体のサブクラスにおいて脂肪酸部分を実質的に除去することにより、疼痛の治療に特に有用な薬物が得られるという予想外の発見に基づいている。このスピカマイシン誘導体のサブクラスは、脂肪酸を含む誘導体に比べて高い水溶性を示し、したがって、ヒトへの投与に適した調合物（例えば、生理的緩衝液を含む調合物）への許容性がより高い。加えて、このスピカマイシン誘導体のサブクラスを含む薬学的調合物は、活性成分を可溶化するために有毒な親油性担体を必要としないため、可能性のある毒性を低下させることができる。

【０００４】

したがって、本発明は、疼痛軽減を必要とする被験者（例えば、ヒト、イヌ、ネコ、またはウマなどの哺乳類）を同定すること；および被験者における著しい疼痛軽減を提供するのに有効な式ＩＩの化合物の一定量を被験者に投与することにより、疼痛軽減を提供する方法を特徴とする。

【化７】



式 II

R_1およびR_2は互いに異なり、－Ｈまたは－ＯＨを表し、かつＲは（１）一つもしくは二つの炭素原子（すなわち、二つ以下の炭素原子）を有する置換もしくは無置換アルキル、または（２）－Ｈを表す。例えば、Ｒはアミノ基（例えば、一級アミノ基）、カルボニル基または両方を含みうる。Ｒが－ＣＯＣＨ$_2$ＮＨ$_2$であり、R_1が－Ｈであり、かつR_2が－ＯＨである場合、化合物は４’－Ｎ－グリシルスピカマイシンアミノヌクレオシド（ＳＡＮ－Ｇｌｙ）として公知である。Ｒが－Ｈである場合、化合物はＳＡＮとして公知である。例えば、カミショウハラ（Ｋａｍｉｓｈｏｈａｒａ）ら、Ｏｎｃｏｌｏｇｙ Ｒ

ｅｓ．６：３８３～３９０、１９９４を参照されたい。式ＩＩの化合物の塩も、本発明の方法において用いることができる。

【０００５】

本明細書の方法において有用な式ＩＩの化合物（およびその塩）は、R_1およびR_2が独立にＨまたはＯＨで、ただしR_1およびR_2は同時に同じではなく、かつ：

各Ｒ基は独立にＨ、または１個から３個の独立のR^3もしくはR^4で置換された１から５個（例えば、１個から２個、１個から３個、１個から４個）の炭素原子を有するアルキル基であってもよく；

各R^3は独立に、いずれも１個～３個の独立のR^5で選択的に置換されたヘテロシクリルまたはヘテロアリールであり；

各R^4は独立にハロゲン、酸素、硫黄、CF_3、SR^6、OR^6、$OC(O)R^6$、NR^6R^6、NR^6R^7、$COOR^6$、$C(O)R^6$、または$C(O)NR^6R^6$であり；

各R^5は独立にＣ１～Ｃ１０アルキル；ハロ；ハロアルキル；SR^6；OR^6；NR^6R^6；$COOR^6$；NO_2；ＣＮ；$C(O)R^6$；$C(O)NR^6R^6$；$OC(O)R^6$；$S(O)_2R^6$；$S(O)_2NR^6R^6$；$NR^6C(O)NR^6R^6$；$NR^6C(O)R^6$；$NR^6(COOR^6)$；$NR^6C(O)R^8$；$NR^6S(O)_2NR^6R^6$；$NR^6S(O)_2R^6$；$NR^6S(O)_2R^8$；またはR^4もしくはR^8で置換されたＣ１～Ｃ１０アルキルであり；

各R^6は独立にＨ、Ｃ１～Ｃ１０アルキル；Ｃ２～Ｃ１０アルケニル；Ｃ２～Ｃ１０アルキニル；Ｃ３～Ｃ１０シクロアルキル；R^8；またはR^8で置換されたＣ１～Ｃ１０アルキルであり；

各R^7は独立に$COOR^9$、$C(O)NR^9R^9$、$S(O)_2R^9$；または$S(O)_2NR^9R^9$であり；

各R^8は独立にアリール、ヘテロアリール、またはヘテロシクリルであり；

各R^9は独立にＨ、Ｃ１～Ｃ１０アルキル、アリール、ヘテロアリール、またはヘテロシクリルである化合物でもある。

【０００６】

「アルキル」という用語は、炭素原子を含む直鎖もしくは分枝炭化水素鎖または環状炭化水素部分を意味する。これらのアルキル基は一つまたは複数の二重結合または三重結合を含んでいてもよい。「置換アルキル」とは、アルキルの原子が、例えば、炭素、窒素、硫黄、酸素、もしくはハロゲン原子で、または窒素、硫黄、酸素、もしくはハロゲン原子で置換されているアルキルを意味する。

【０００７】

「置換アルキル」におけるアルキル基の任意の原子に結合されうる置換基の例には、ヘテロシクリル基；ヘテロアリール基、アミノ基、アミド基、アルコキシ基、アシルオキシ基、チオアルコキシ基、アシルチオアルコキシ基、ハロゲン基、スルホネート基、スルホンアミド基、エステル基、カルボン酸、酸素（例えば、カルボニル基）および硫黄（例えば、チオカルボニル基）が含まれる。置換基には分子の水溶性を改善するいかなる化学官能基（例えば、カルボン酸、カルボン酸エステル、カルボキサミド、モルホリノ、ピペラジニル、イミダゾリル、チオモルホリノ、またはテトラゾリル基；置換および無置換の両方）も含まれる。

【０００８】

「ハロ」および「ハロゲン」なる用語は、フッ素、塩素、臭素またはヨウ素のいかなる基も意味する。「環」および「環構造」なる用語は、表示の数の原子を含み、該原子は炭素、または示されている場合には窒素、酸素もしくは硫黄などのヘテロ原子である環を意味する。環自体、ならびにその上の任意の置換基が、安定な化合物の形成を可能にする任意の原子に結合されていてもよい。

【０００９】

「アリール」なる用語は、６－炭素単環式または１０－炭素二環式芳香環構造であって、ここで各環の０、１、２または３個の原子は置換基で置換されていてもよい環構造を意味

する。アリール基の例にはフェニル、ナフチルなどが含まれる。

【００１０】

「ヘテロアリール」なる用語は、芳香族の５員～８員単環式、８員～１２員二環式、または１１員～１４員三環式環構造であって、単環式であれば１個～３個のヘテロ原子、二環式であれば１個～６個のヘテロ原子、または三環式であれば１個～９個のヘテロ原子を含み、該ヘテロ原子はＯ、Ｎ、またはＳから選択され、各環の０、１、２または３個の原子は置換基で置換されていてもよい環構造を意味する。ヘテロアリール基の例には、ピリジル、フリルまたはフラニル、イミダゾリル、ベンズイミダゾリル、ピリミジニル、チオフェニルまたはチエニル、キノリニル、インドリル、チアゾリルなどが含まれる。

【００１１】

「ヘテロシクリル」なる用語は、非芳香族の５員～８員単環式、８員～１２員二環式、または１１員～１４員三環式環構造であって、単環式であれば１個～３個のヘテロ原子、二環式であれば１～６個のヘテロ原子、または三環式であれば１個～９個のヘテロ原子を含み、該ヘテロ原子はＯ、Ｎ、またはＳから選択され、各環の０、１、２または３個の原子は置換基で置換されていてもよい環構造を意味する。ヘテロシクリル基の例には、ピペリジニル、ピロリジニル、ジオキサニル、モルホリニル、テトラヒドロフラニルなどが含まれる。

【００１２】

本発明によって企図される置換基および変量の組み合わせは、結果として安定な化合物を形成するものだけである。本明細書において用いられる「安定な」なる用語は、製造を可能にするのに十分な安定性を有し、かつ本明細書において詳述される目的（例えば、被験者への治療的もしくは予防的投与、または防腐薬、創傷包帯含浸、滅菌剤、もしくは消毒剤への適用）のために有用たるに十分な期間、化合物の完全性を維持する化合物を意味する。

【００１３】

「疼痛軽減を必要とする被験者」は、必ずしも現在疼痛を経験しているわけではなく、「疼痛軽減」は１００％未満の疼痛低減を含む。例えば、本発明は、いかなる原因による神経障害性疼痛、例えば、ヘルペス後神経痛、幻肢痛または切断端痛、糖尿病性神経障害、後天性免疫不全症候群神経障害、背痛、および内臓痛（例えば慢性膵炎）に対しても、ヒト患者、イヌ、ネコ、またはウマを含む哺乳類を治療するために用いることができる。「神経障害性疼痛」とは、末梢神経系への損傷または末梢神経系の障害によって生じる疼痛を意味する。

【００１４】

化合物は局所または全身、例えば、インプラント（例えば、遅延放出のため）を介して、または静脈内大量注射もしくは塊状注入により投与することができる。「インプラント」とは、皮膚よりも深部の組織内にある任意の装置であって、その組織内で装置は化合物を制御放出または持続放出する。そのような装置は薬物送達の分野では公知である（例えば、米国特許第６，０１３，８５３号参照）。例えば、式ＩＩの化合物を、マイクロカプセル送達系を含む、制御放出調合物などの体内からの急速な排出から化合物を保護する担体と共に調製することができる。エチレン酢酸ビニル、ポリ無水物、ポリグリコール酸、コラーゲン、ポリオルトエステル、およびポリ乳酸などの生物分解性、生物適合性ポリマーを用いることができる。そのような調合物の調製法は、当業者には明らかであると思われる。この物質は、アルザ社（Ａｌｚａ Ｃｏｒｐｏｒａｔｉｏｎ）およびノバ ファーマシューティカルズ社（Ｎｏｖａ Ｐｈａｒｍａｃｅｕｔｉｃａｌｓ，Ｉｎｃ．）からも市販されている。リポソーム懸濁液も、薬学的に許容される担体として用いることができる。これらは、例えば米国特許第４，５２２，８１１号に記載のとおり、当業者には公知の方法に従って調製することができる。一回で投与される化合物の量は、体表面積１ｍ^{2}あたり約１ｎｇから４ｍｇ（例えば、体表面積１ｍ^{2}あたり８０ｎｇから１ｍｇ）であってもよく、化合物は薬学的に許容される担体を選択的に含む水溶液に調合することができる。他の適当な用量は、体重１ｋｇあたり約１ｍｇから１０００ｍｇ（例えば、体重１ｋｇ

あたり約１０ｍｇから５００ｍｇ、または約１００ｍｇ）を含む。

【００１５】

本発明に従った治療は、現在の疼痛が、オピオイド薬物の使用などの他の疼痛軽減法に抵抗性である患者で、疼痛の軽減をもたらす。本発明は、疼痛を予防するために、疼痛を予測して用いることもできる。

【００１６】

本発明はさらに、選択的に急性侵害受容性疼痛よりも神経障害性疼痛を選択的に阻害、治療、または予防する方法であって、疼痛軽減を必要とする被験者を同定する段階、および式ＩＩのものを含む、本明細書に示されている任意の式の化合物（または組成物）を投与する段階を含む方法に関する。もう一つの局面において、その方法は選択的に侵害受容性疼痛よりも神経障害性疼痛を選択的に阻害、治療、または予防することを含み、疼痛軽減を必要とする被験者を同定する段階、および本明細書に示されている任意の式の化合物（または組成物）を投与する段階を含む。これらの方法の一つの局面において、選択的にとは、侵害受容性疼痛よりも神経障害性疼痛をより強く阻害することを意味する。これらの方法のもう一つの局面において、選択的にとは、１９９９年５月１８日発行のボルスーク（Ｂｏｒｓｏｏｋ）ら、米国特許第５，９０５，０６９号（およびその中で引用されている参考文献）、およびアブディ（Ａｂｄｉ）ら、Ａｎｅｓｔｈ．Ａｎａｌｇ．９１、９５５～９９９（２０００）に記載のものを含む、標準疼痛モデルによって決定されるとおり、侵害受容性疼痛よりも神経障害性疼痛を少なくとも５０％強く（例えば、＞１００％強く、＞２００％強く、５００％強く）阻害することを意味する。

【００１７】

本発明は、神経障害性疼痛治療を含む、疼痛仲介に関与する受容体として、遺伝子（またはそれらの遺伝子によってコードされるポリペプチド）を査定、同定、または有効性確認する方法にも関する。この方法は、本明細書における任意の式の化合物を被験ポリペプチドと接触させる段階、およびその化合物と被験ポリペプチドとの結合親和性を測定する段階を含む。化合物に対してより大きい親和性を有するポリペプチド（およびそれらをコードする遺伝子）が、疼痛仲介、特に神経障害性疼痛の仲介に直接関与する可能性がより大きい候補である。したがって、これらは、疼痛の治療または予防のための、疼痛仲介の新しい機構およびリガンド（例えば、ペプチドまたは小分子薬物）による阻害の標的についての調査および開発研究の興味深い標的であると考えられる。化合物と受容体との結合親和性の解析は、化合物、標的、もしくは既知のペプチドーリガンド相互作用を仲介する他のリガンドを検出するための標識（放射性標識、蛍光）研究を含む、薬物スクリーニング／設計、ゲノム科学、および医薬品化学分野において公知のアッセイ法、方法、および技術を用いて実施することができるか、または間接的読出し（例えば、その放出または生成が化合物と被験ポリペプチドとの結合相互作用に依存しているマーカーの存在を測定する）を含むこともできる。

【００１８】

本発明はさらに、疼痛薬として可能性があるもの（例えば、ペプチド、化学的実体、小分子）の効力を査定する方法にも関する。本明細書における式の化合物は神経障害性疼痛の仲介において有効であるため、これらは新しい疼痛薬として可能性があるものを査定することができる「標準」としても有用である。そのような方法は、被験化合物（例えば、疼痛薬として可能性があるもの）および式ＩＩの化合物を含む本明細書における任意の式の化合物（すなわち標準）を、被験化合物および本明細書における任意の式の化合物の、疼痛仲介または疼痛の機構調節における有効性の尺度または査定を提供する被験者または媒体（例えば、患者、動物モデル、細胞培養、インビトロアッセイ法）に供することにより、被験化合物を本明細書における任意の式の化合物に対して評価する段階を含む。この方法は、被験化合物の疼痛薬としての有効性を査定するために、化合物試験の結果を評価する段階をさらに含んでいてもよい。これらの方法における化合物の有効性の測定または査定は、当技術分野において公知で、かつ容易に利用可能な任意の数の適当な技術およびプロトコルによって実施することができる。

【００１９】
本発明は、疼痛仲介に関与する新しい遺伝子、受容体、またはペプチドを同定する方法にも関する。これらの方法は、式ＩＩの化合物を含む、本明細書における任意の式の化合物によって引き出される、疼痛仲介に関与する効果を含む代謝効果を調べるための、これらの化合物の使用を含む。そのような方法は、本明細書における任意の式の化合物を、被験者または媒体（例えば、患者、動物モデル、細胞培養、インビトロアッセイ法、組織）に投与する段階、被験者または媒体から組織（例えば、背根神経節、神経組織、脊髄組織、または中枢神経系（ＣＮＳ）組織）を採取する段階、および公知または新規の遺伝子、受容体、またはペプチドの効果について組織を評価する（例えば、誘導、抑制、間接的応答、マーカー産生を同定および／または定量する）段階を含む。公知または新規の遺伝子、受容体、またはペプチドは、疼痛を仲介する新しい薬物のための興味深い標的であり、新しい疼痛仲介、治療、または予防法のための新しい情報および新規な標的を提供する。これらの方法における評価は、遺伝子、受容体、またはペプチドを測定、検出、および同定するための、当技術分野において公知で、かつ容易に利用可能な任意の数の適当な技術およびプロトコルを用いて実施することができる。

【００２０】
別に定義されていない限り、本明細書において用いられるすべての科学・技術用語は、本発明が属する分野の当業者によって一般に理解されているものと同じ意味を持つ。適当な方法および材料が以下に記載されているが、本発明の実施または試験において、本明細書に記載のものと類似または同等の方法および材料を用いることもできる。本明細書において言及されるすべての刊行物、特許出願、特許、および他の参考文献は、その全体が参照として本明細書に組み入れられる。抵触がある場合には、定義を含む本明細書により規制される。加えて、材料、方法、および実施例は単なる例示であって、限定するためのものではない。

【００２１】
本発明の他の特徴および利点は、下記の詳細な説明および特許請求の範囲から明らかになると思われる。

【００２２】
詳細な説明

本発明は、被験者に疼痛を低減または予防するのに十分な量の水溶性スピカマイシン誘導体（式ＩＩ）を投与することにより、疼痛を低減または予防する方法に関する。したがって、本発明の化合物は、任意の適当な経路、例えば、静脈内、動脈内、局所、鼻腔内、吸入により肺内、腹腔内、胸膜内、経口、皮下、筋肉内、舌下、表皮内、膣内、または直腸内により投与することができる。化合物は液剤、懸濁剤、坐剤、錠剤、顆粒剤、散剤、カプセル剤、軟膏、またはクリーム剤として調合することができる。溶媒（例えば、水または生理食塩水）、可溶化剤（例えば、エタノール、ポリソルベート、またはクレモフォアＥＬ７（Ｃｒｅｍｏｐｈｏｒ ＥＬ７：登録商標）、等張化剤、保存剤、抗酸化剤、賦形剤（例えば、乳糖、デンプン、結晶性セルロース、マンニトール、麦芽糖、リン酸水素カルシウム、軽質無水ケイ酸、または炭酸カルシウム）、結合剤、（例えば、デンプン、ポリビニルピロリドン、ヒドロキシプロピルセルロース、エチルセルロース、カルボキシメチルセルロース、またはアラビアガム）、滑沢剤（例えば、ステアリン酸マグネシウム、タルク、または硬化油）、または安定化剤（例えば、乳糖、マンニトール、麦芽糖、ポリソルベート、マクロゲル、またはポリオキシエチレン硬化ヒマシ油）などの様々な添加剤をこれらの調合物に加えることもできる。適当であれば、下記の化合物も加えることができる：グリセリン、ジメチルアセトアミド、乳酸ナトリウム、界面活性剤、または水酸化ナトリウム、エチレンジアミン、エタノールアミン、重炭酸ナトリウム、アルギニン、メグルミン、もしくはトリスアミノメタンなどの塩基性物質。前述のとおり、式ＩＩの化合物を含む薬学的調合物に有機溶媒（例えば、エタノール）は必要ではない。しかし、疎水性材料（例えば、第二の鎮痛剤）が調合物に含まれる場合、または調合物の薬物動態特性を調節すべき場合には、前掲の可溶化剤および有機物質を用いることができる。液剤、錠

剤、顆粒剤、またはカプセル剤などの薬学的調製物を、これらの成分などと共に形成することができる。

【００２３】

本発明の化合物の用量は、動物実験の結果および様々な条件を考慮して決定される。例えば、いかなる疼痛軽減候補化合物も、下記の実施例に記載の動物モデルで試験することができる。より具体的な用量は明らかに、投与法や、年令、体重、性別、感受性、摂取した食事、投与間隔、組み合わせて投与された薬剤、ならびに疼痛の原因、重症度、および程度などの被験者の条件に応じて変動する。所与の条件下での最適用量および投与頻度は、前述の指針に基づいて、医療専門家の適当な用量試験によって決定しなければならない。

【００２４】

ＳＡＮおよびＳＡＮ－Ｇｌｙなどの、水溶性、非毒性、または低毒性のスピカマイシン誘導体は、当技術分野において公知の方法を用いて調製することができる。例えば、一般的合成方法は米国特許第５，４６１，０３６号および第５，６３１，２３８号に記載されている。これらの戦略を適合させて、式ＩＩに示すとおり、一つまたは二つの炭素を含む任意のＲ基を糖基に結合することができる。ＳＡＮおよびＳＡＮ－Ｇｌｙを調製するための具体的な半合成戦略は、カミショウハラ（Ｋａｍｉｓｈｏｈａｒａ）ら、Ｊ．Ａｎｔｉｂｉｏｔｉｃｓ ４６：１４３９～１４４６、１９９３；カミショウハラ（Ｋａｍｉｓｈｏｈａｒａ）ら、Ｏｎｃｏｌｏｇｙ Ｒｅｓ．６：３８３～３９０、１９９４；ならびに米国特許第５，４６１，０３６号および第５，６３１，２３８号に記載されている。本明細書に記載の阻害剤化合物を合成する際に有用な合成化学的変換および保護基方法論（保護と脱保護）は当技術分野において公知であり、例えば、Ｒ．ラロック（Ｒ．Ｌａｒｏｃｋ）、「総合有機変換（Ｃｏｍｐｒｅｈｅｎｓｉｖｅ Ｏｒｇａｎｉｃ Ｔｒａｎｓｆｏｒｍａｔｉｏｎｓ）」、ＶＣＨ Ｐｕｂｌｉｓｈｅｒｓ（１９８９）；Ｔ．Ｗ．グリーン（Ｔ．Ｗ．Ｇｒｅｅｎｅ）およびＰ．Ｇ．Ｍ．ビュッツ（Ｐ．Ｇ．Ｍ．Ｗｕｔｓ）、「有機合成における保護基（Ｐｒｏｔｅｃｔｉｖｅ Ｇｒｏｕｐｓ ｉｎ Ｏｒｇａｎｉｃ Ｓｙｎｔｈｅｓｉｓ）」、第２版、Ｊｏｈｎ Ｗｉｌｅｙ ａｎｄ Ｓｏｎｓ（１９９１）；Ｌ．フィーザー（Ｌ．Ｆｉｅｓｅｒ）およびＭ．フィーザー（Ｍ．Ｆｉｅｓｅｒ）、「フィーザーおよびフィーザーの有機合成用試薬（Ｆｉｅｓｅｒ ａｎｄ Ｆｉｅｓｅｒ’ｓ Ｒｅａｇｅｎｔｓ ｆｏｒ Ｏｒｇａｎｉｃ Ｓｙｎｔｈｅｓｉｓ）」、Ｊｏｈｎ Ｗｉｌｅｙ ａｎｄ Ｓｏｎｓ（１９９４）；およびＬ．パケット（Ｌ．Ｐａｑｕｅｔｔｅ）編、「有機合成用試薬百科事典（Ｅｎｃｙｃｌｏｐｅｄｉａ ｏｆ Ｒｅａｇｅｎｔｓ ｆｏｒ Ｏｒｇａｎｉｃ Ｓｙｎｔｈｅｓｉｓ）」、Ｊｏｈｎ Ｗｉｌｅｙ ａｎｄ Ｓｏｎｓ（１９９５）、ならびにそれぞれその後の版に記載のものなどが含まれる。

【００２５】

本発明の化合物はそのすべての塩を含む。そのような塩の例には、薬学的に許容される無機および有機酸および塩基由来のものが含まれる。適当な酸性塩の例には、酢酸塩、アジピン酸塩、アルギン酸塩、アスパラギン酸塩、安息香酸塩、酪酸塩、クエン酸塩、フマル酸塩、グリコール酸塩、ヘミ硫酸塩、ヘプタン酸塩、ヘキサン酸塩、塩酸塩、臭化水素酸塩、ヨウ化水素酸塩、２－ヒドロキシエタンスルホン酸塩、乳酸塩、マレイン酸塩、マロン酸塩、メタンスルホン酸塩、ニコチン酸塩、硝酸塩、シュウ酸塩、パルモ酸塩、ペクチン酸塩、過硫酸塩、ピクリン酸塩、ピバル酸塩、プロピオン酸塩、サリチル酸塩、コハク酸塩、硫酸塩、酒石酸塩、チオシアン酸塩、トシル酸塩およびウンデカン酸塩が含まれる。シュウ酸などの他の酸は、それ自体では薬学的に許容されないが、本発明の化合物およびそれらの薬学的に許容される酸付加塩を得る際の中間体として有用な塩の調製において用いることができる。適当な塩基由来の塩には、アルカリ金属（例えばナトリウム、カリウム）、アルカリ土類金属（例えばマグネシウム）、アンモニウムおよびＮ－（アルキル）$_4^+$塩が含まれる。本明細書における式の化合物には、その中の任意の塩基性窒素含有基の四級化を有するものが含まれる。本発明の化合物は一つまたは複数の不斉中心を含んでいてもよく、したがってラセミ体およびラセミ混合物、単一の鏡像異性体、個々のジアステレオマーならびにジアステレオマー混合物として出現する。これらの化合物のそのよ

うな異性体はすべて明らかに本発明に含まれる。

【００２６】

神経障害性疼痛とは、末梢神経系の損傷または障害由来の疼痛である（ウールフ（Ｗｏｏｌｆ）、Ａｃｔａ Ｎｅｕｒｏｃｈｉｒ．５８：１２５～１３０、１９９３、およびベネット（Ｂｅｎｎｅｔｔ）、「疼痛教本（Ｔｅｘｔｂｏｏｋ ｏｆ Ｐａｉｎ）」における「神経障害性疼痛（Ｎｅｕｒｏｐａｔｈｉｃ Ｐａｉｎ）」、Ｐ．Ｄ．ウォール（Ｐ．Ｄ．Ｗａｌｌ）およびＲ．マルザック（Ｒ．Ｍａｌｚａｃｋ）編、２０１～２２４、Ｃｈｕｒｃｈｉｌｌ Ｌｉｖｉｎｇｓｔｏｎｅ、エジンバラ（１９９４）に総説掲載）。神経障害性疼痛を有する患者は典型的には、持続的熱傷もしくは灼熱痛、感覚の部分的消失、触覚もしくは寒冷異痛症、または反復刺激に対する痛覚過敏を含む、原因に無関係の特徴的な一連の感覚障害を示す。末梢神経障害性疼痛にはいくつかの異なる状態が含まれ、そのうち最も一般的であるのは、三叉神経痛、ヘルペス後神経痛、有痛性糖尿病神経障害、ならびに灼熱痛、単神経障害、および末梢神経損傷を含む反射交感神経ジストロフィである。

【００２７】

侵害受容性疼痛とは、神経系外の損傷または疾患が原因の疼痛である。これは神経障害性疼痛に特徴的な鋭い、外傷様の疼痛ではなく、むしろ進行中の鈍い痛みまたは圧迫であることが多い。侵害受容性疼痛の例には、癌または関節炎、捻挫、骨折、熱傷、こぶ、挫傷による疼痛が含まれる。急性疼痛では、疼痛の重症度は組織損傷の程度に直接相関する。これにより、鋭利または熱い物にふれた場合、ただちに手を動かす反射などの防衛反射が起こる。この型の疼痛は損傷または患部組織の症状であるため、根元的問題が治癒すれば疼痛も消失する。慢性疼痛では、疼痛は防衛または他の生物学的機能を果たしていないため、急性疼痛とは異なる。むしろ、神経は持続的組織損傷がなくても、脳に痛みのメッセージを送り続ける。

【００２８】

疼痛の機構および型が異なるため、選択的に侵害受容性疼痛よりも神経障害性疼痛を選択的に治療または予防することが有益でありうる。別の場合には、疼痛または疼痛症状は神経障害性と侵害受容性の両方の組み合わせであることもあり、そのような場合、両方の型の疼痛の治療が適当でありうる。

【００２９】

オピオイド薬物に抵抗性の無能化疼痛を有する患者に対し、非外科的代替法はほとんどない。本発明の方法は、そのような患者に代替の水溶性疼痛軽減剤を提供する。水溶性が高くなれば、被験者に経口投与した場合に、全身循環への吸収が高まり、それによって薬物の生物学的利用能が高まりうる。

【００３０】

加えて、ＳＡＮ－Ｇｌｙなどの水溶性スピカマイシン誘導体は、ＫＲＮ５５００（カミショウハラ（Ｋａｍｉｓｈｏｈａｒａ）ら、Ｏｎｃｏｌｏｇｙ Ｒｅｓ．６：３８３～３９０、１９９４）などの水不溶性スピカマイシン誘導体よりも細胞毒性が低い。このことは、水溶性スピカマイシン誘導体は、慢性疼痛を治療するために必要となりうる反復投与を受ける患者にとって、より安全である。

【００３１】

本発明は、水溶性スピカマイシン誘導体および第二の鎮痛剤または抗炎症剤などの薬物（例えば、アスピリン、アセトアミノフェン、イブプロフェン、ナプロキセン、ジクロフェナク、セレコキシブ、ＮＳＡＩＤＳ、ＣＯＸ－１阻害剤、ＣＯＸ－２阻害剤、ステロイド、ステロイド誘導体、グルココルチコイド）を含む組み合わせ調合物も企図する。

【００３２】

これ以上詳細な記載がなくとも、当業者であれば前述の開示および以下の説明に基づき、本発明を完全に利用することができると考えられる。以下の実施例は、当業者がいかにして本発明を実施しうるかを単に例示するためのものと解釈されるべきであり、開示の他の部分をいかなる様式でも制限するものではない。本開示に引用されるすべての特許および

刊行物は、参照として本明細書に組み入れられる。

【００３３】

実施例１

比較的水溶性で、低毒性のスピカマイシン誘導体が疼痛軽減に有用であるかどうかを調べるために、化合物ＳＡＮ－Ｇｌｙを、米国特許第５，６３１，２３８号に記載のとおり半合成的に調製した。次いで、ＳＡＮ－Ｇｌｙを食塩水中で調合した。

【００３４】

体重１５０ｇ～２００ｇの雄Ｓｐｒａｇｕｅ－Ｄａｗｌｅｙラット（Ｃｈａｒｌｅｓ Ｒｉｖｅｒ Ｌａｂｏｒａｔｏｒｉｅｓ）を用いて、ＳＡＮ－Ｇｌｙ調合物の鎮痛性を評価した。ラットを３匹の群で、軟らかい床敷きを備えたプラスチックケージ内で、１２時間の明暗サイクルで飼育した。飼料および水は自由に与えた。１週間の実験室条件への馴化後、基準の機械的異痛症を確立するため、すべてのラットを試験した。

【００３５】

異なる動物疼痛モデルは異なる実験結果を生じうるため、ＳＡＮ－Ｇｌｙを二つのラットモデルを用いて試験した。すべての実験は一重盲検様式、すなわち実験者はどのラットが対照食塩水の投与を受け、どのラットがＳＡＮ－Ｇｌｙ調合物の投与を受けるか知らない状態で実施した。

【００３６】

用いた一つのラットモデルは、実験的に誘発した分節脊髄神経損傷（キム（Ｋｉｍ）ら、Ｐａｉｎ ５０：３５５～３６３、１９９２）に基づいていた。実験室条件への馴化の１週間後、基準測定を記録し、キムら、上記に記載のとおりに手術を行った。ラットを酸素中のハロタンで麻酔し、腹臥位に置いた。Ｌ４～Ｓ２での正中線皮膚切開を行い、Ｌ４～Ｓ２領域で傍脊柱筋を棘突起から分離した。左Ｌ５およびＬ６脊髄神経を同定し、６－Ｏ絹糸できつく結紮した。次いで、創傷を縫合した。手術終了時、麻酔を中止し、ラットをケージに戻して、飼料ペレットおよび水を自由に与えた。ラットは約１０分以内に麻酔から回復した。

【００３７】

ラットを、少なくとも１週間、手術から回復させた。手術の１０日から１２日後、ホンフライ毛（ＶＦＦ）による刺激に対する過敏性をモニターすることにより、ラットの異痛症を試験した。ラットを網の床に置き、底の開いた透明なプラスチックの箱で覆いをした。較正したＶＦＦ（３．６１、３．８４、４．０８、４．３１、４．５６、４．７４、４．９３、および５．１６）を左後足の足底皮膚に上げ下げ法を用いて適用し、５０％の足の引っ込めを疼痛閾値とした。各ＶＦＦをラットの下から、網の床を通して挿入し、毛がちょうど曲がるまで足の第二、第三、および第四指に適用することにより試験した（チャプラン（Ｃｈａｐｌａｎ）ら、Ｊ．Ｎｅｕｒｏｓｃｉ．Ｍｅｔｈｏｄｓ ５３：５５～６３、１９９４）。試験はＶＦＦ適用を４回反復して行った（１０秒～１５秒に１回の頻度）。次いで、ラットを二群に分け、一群には食塩水を投与し、もう一群にはＳＡＮ－Ｇｌｙ調合物（体重１ｋｇあたり１００ｍｇ；尾静脈に一回の大量注入）を投与した。次いで、両群を手術後の様々な時点で異痛症についてモニターした。

【００３８】

他のラットモデルは、実験的に誘発した慢性締め付け神経損傷（ｃｈｒｏｎｉｃ ｃｏｎｓｔｒｉｃｔｉｏｎ ｎｅｒｖｅ ｉｎｊｕｒｙ）（ベネット（Ｂｅｎｎｅｔｔ）ら、Ｐａｉｎ ３３：８７～１０７、１９８８）に基づいていた。ベネットら、上記に記載のとおりに、ラットで末梢単神経障害を引き起こした。ハロタン麻酔下でラットに手術を行い、総坐骨神経を露出した。次いで、神経を緩く結紮し、創傷を縫合し、ラットを少なくとも１週間回復させた。次いで、ラットの異痛症を試験し、二群に分け、前述のとおりに再度試験した。

【００３９】

両方の動物モデルを用いた結果を合わせ、図１に概要を示している。ＳＡＮ－Ｇｌｙの単回注入により、基準疼痛行動への６０％の回復が得られ、この疼痛耐容性上昇は手術後少

なくとも７日間持続した。したがって、ＳＡＮ－Ｇｌｙは二つの動物疼痛モデルで鎮痛剤として有用であることが判明した。

【００４０】

実施例２

９５％ O_2 および５％ CO_2 飽和氷冷クレブス液（ＮａＣｌ １１７ｍＭ、ＫＣｌ ３．６ｍＭ、$CaCl_2$ ２．５ｍＭ、$MgCl_2$ １．２ｍＭ、NaH_2PO_4 １．２ｍＭ、$NaHCO_3$ ２５ｍＭおよびグルコース１１ｍＭ）で灌流したＬ４背根付きの、厚さ６５０μｍの腰髄横断切片（残存神経損傷（ｓｐａｒｅｄ－ｎｅｒｖｅ ｉｎｊｕｒｙ）動物から）で電気生理学的記録を行った。ＩＩ層からの一次求心線維誘発性の興奮性シナプス後電流（ＥＰＳＣ）を記録するために、全細胞をパッチクランプし、次いで－７０ｍＶで電位固定した。Ｌ４背根の順方向性刺激を、定電流刺激装置に接続された吸引電極で実施し、異なる求心線維群（Ａβ、Ａδ、およびＣ線維）を漸増するのに十分な段階的強度の刺激を用いた。パッチピペットの抵抗は Cs_2SO_4 １１０ｍＭ、$CaCl_2$ ０．５ｍＭ、$MgCl_2$ ２ｍＭ、ＥＧＴＡ ５ｍＭ、ＨＥＰＥＳ ５ｍＭ、ＴＥＡ ５ｍＭ、ＡＴＰ－Ｍｇ塩 ５ｍＭで充填した場合に５ＭＷ～１０ＭＷであった。電流を増幅し（Ａｘｏｐａｔｃｈ ２００Ａ）、２ｋＨｚでフィルターにかけ、５ｋＨｚでデジタル化し、ｐＣＬＡＭＰ６（Ａｘｏｎ Ｉｎｓｔｒｕｍｅｎｔｓ）を用いて解析した。ＳＡＮ－Ｇｌｙを二段階の濃度（１０ｍＭおよび１００ｍＭ）で氷冷クレブス液に溶解し、氷浴を適用した。５つの細胞のうちの３つで、１００ｍＭのＳＡＮ－ＧｌｙがＥＰＳＣの潜時を増大させ、振幅を減少させることが観察され、この化合物が後角のシナプス伝達阻害において役割を果たしているとの仮説と一致した。この効果は薬物の洗浄によって逆転した。加えて、予想通り、同じ濃度のＳＡＮはまったく効果がなかった。この実施例は、ＳＡＮ－Ｇｌｙおよびその誘導体が神経障害性疼痛を治療する際に有用であることを示している。

【００４１】

他の態様

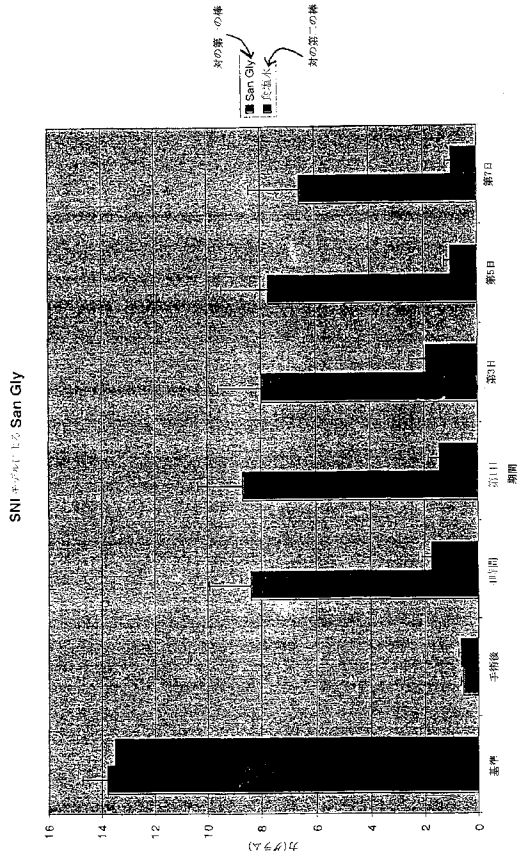
本発明はその詳細な説明に関して記載されているが、前述の説明は例示のためのもので、本発明の範囲を限定するものではなく、本発明は添付の特許請求の範囲によって規定されることが理解されるべきである。他の局面、利点、および変更は本発明の範囲内である。

【図面の簡単な説明】

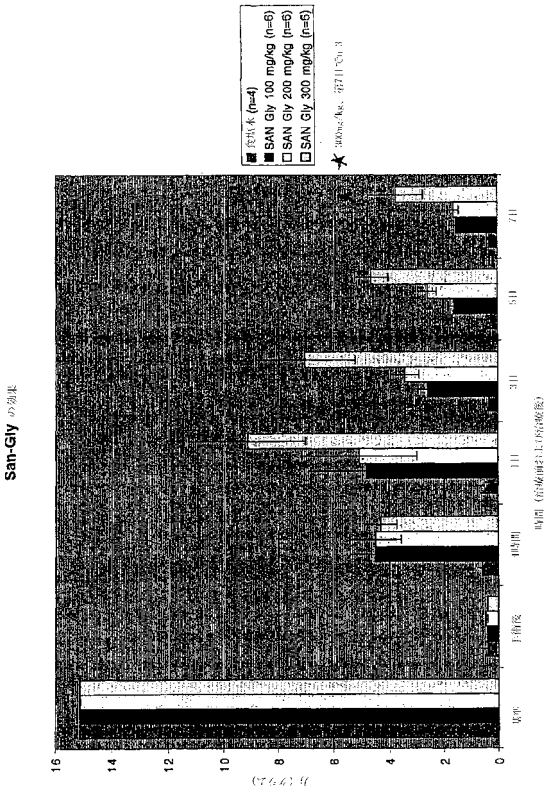
【図１】未治療ラットおよびＳＡＮ－Ｇｌｙで治療したラットでの、試験時間に対する力（グラム）（高い力は高い疼痛閾値に相関する）の棒グラフである。誤差バーは１つの標準偏差を示す。

【図２】未治療ラットならびに１００ｍｇ／Ｋｇ、２００ｍｇ／Ｋｇ、および３００ｍｇ／ＫｇのＳＡＮ－Ｇｌｙで治療したラットでの、試験時間に対する力（グラム）（高い力は高い疼痛閾値に相関する）の棒グラフである。誤差バーは１つの標準偏差を示す。

【図１】


SNI
San Gly
Sal Gly
基準
手術後
第1日
第3日
第5日
第7日
期間

【図２】


San-Gly
SAN Gly 100 mg/kg (n=6)
SAN Gly 200 mg/kg (n=6)
SAN Gly 300 mg/kg (n=6)
基準
手術後
期間

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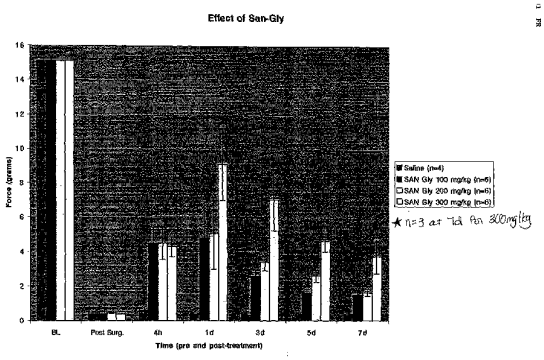
(54) Title: METHODS OF DECREASING OR PREVENTING PAIN USING SPICAMYCIN DERIVATIVES



(57) Abstract: Methods of providing pain relief by administering a water-soluble derivative of spicamycin. Methods of using pain mediation agents are also provided.

WO 02/24146 A2

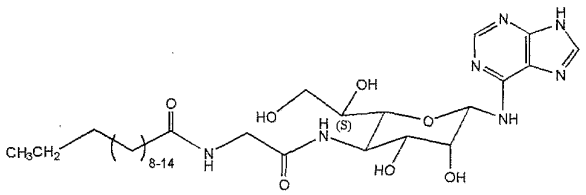
METHODS OF DECREASING OR PREVENTING PAIN USING SPICAMYCIN DERIVATIVES

Cross-Reference to Related Applications

This application claims the benefit of U.S. provisional application 60/234,382, filed September 20, 2000.

Background of the Invention

Spicamycin (SPM) is an antitumor antibiotic produced by the bacterium *Streptomyces*



alanosinicus 879-MT_3 (Hayakawa et al., Agric. Biol. Chem. 49:2685-2691, 1985). Spicamycin and its derivatives are also used for pain relief (U.S. Patent No. 5,905,069). Naturally occurring SPM has the following general structure, varying solely in the fatty acid moiety:

Formula I: Spicamycin

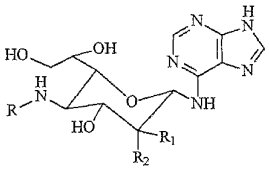
Synthetic variants of spicamycin and their use as an antitumor agent are described in Otake et al., U.S. Patent Nos. 5,461,036 and 5,631,238.

Summary of the Invention

The invention is based on the unexpected discovery that substantial removal of the fatty acid moiety in a subclass of spicamycin derivatives results in drugs especially useful for the treatment of pain. This subclass of spicamycin derivatives exhibit increased water-solubility relative to fatty acid-containing derivatives, and are therefore more amenable to

formulations suitable for human administration (e.g., formulations containing an aqueous physiological buffer). In addition, the potential toxicity of pharmaceutical formulations containing this subclass of spicamycin derivatives can be reduced because no toxic lipophilic carriers are needed in order to solubilize the active ingredients.

Thus, the invention features a method of providing pain relief by identifying a subject (e.g., a mammal, such as a human, dog, cat, or horse) in need of pain relief, and administering to the subject an amount of a compound of Formula II effective to provide significant pain relief in the subject.



Formula II

R_1 and R_2 are different from each other and represent —H or —OH, and R represents (1) a substituted or unsubstituted alkyl having one or two carbon atoms (i.e., no more than two carbon atoms), or (2) —H. For example, R can contain an amino group (e.g., a primary amino group), a carbonyl group or both. When R is $—COCH_2NH_2$, R_1 is —H, and R_2 is —OH, the compound is known as 4'-N-glycyl spicamycin amino nucleoside (SAN-Gly). When R is —H, the compound is known as SAN. See, e.g., Kamishohara et al., Oncology Res. 6:383-390, 1994. A salt of the compound of Formula II can also be used in the methods of the invention.

The compounds (and salts thereof) of Formula II useful in the methods herein also are those where R_1 and R_2 are independently H or OH, wherein R_1 and R_2 are not simultaneously the same and:

Each R group can independently be an H or alkyl group having 1 to 5 (e.g., 1 to 2, 1 to 3, 1 to 4) carbon atoms substituted with 1 to 3 independent R^3 or R^4;

Each R^3 is independently heterocyclyl or heteroaryl, either optionally substituted with 1-3 independent R^5;

Each R^4 is independently halogen, oxygen, sulfur, CF_3, SR^6, OR^6, $OC(O)R^6$, NR^6R^6, NR^6R^7, $COOR^6$, $C(O)R^6$, or $C(O)NR^6R^6$;

Each R^5 is independently C1-C10 alkyl; halo; haloalkyl; SR^6; OR^6; NR^6R^6; $COOR^6$; NO_2; CN; $C(O)R^6$; $C(O)NR^6R^6$; $OC(O)R^6$; $S(O)_2R^6$; $S(O)_2NR^6R^6$; $NR^6C(O)NR^6R^6$; $NR^6C(O)R^6$; $NR^6(COOR^6)$; $NR^6C(O)R^8$; $NR^6S(O)_2NR^6R^6$; $NR^6S(O)_2R^6$; $NR^6S(O)_2R^8$; or C1-C10 alkyl substituted with R^4 or R^8;

Each R^6 is independently H, C1-C10 alkyl; C2-C10 alkenyl; C2-C10 alkynyl; C3-C10 cycloalkyl; R^8; or C1-C10 alkyl substituted with R^8;

Each R^7 is independently $COOR^9$, $C(O)NR^9R^9$, $S(O)_2R^9$; or $S(O)_2NR^9R^9$;

Each R^8 is independently aryl, heteroaryl, or heterocyclyl;

Each R^9 is independently H, C1-C10 alkyl, aryl, heteroaryl, or heterocyclyl.

The term "alkyl" denotes a straight or branched hydrocarbon chain containing carbon atoms or cyclic hydrocarbon moieties. These alkyl groups may also contain one or more double bonds or triple bonds. By "substituted alkyl" is meant an alkyl in which an atom of the alkyl is substituted with, for example, a carbon, nitrogen, sulfur, oxygen, or halogen atom, or alternatively a nitrogen, sulfur, oxygen, or halogen atom.

Examples of substituents that can be attached to any atom of the alkyl group in a "substituted alkyl" include heterocyclyl groups; heteroaryl groups, amino groups, amido groups, alkoxy groups, acyloxy groups, thioalkoxy groups, acyl thioalkoxy groups, halogen groups, sulfonate groups, sulfonamide groups, ester groups, carboxylic acids, oxygen (e.g., a carbonyl group) and sulfur (e.g. a thiocarbonyl group). Substituents also include any chemical functional group that imparts improved water-solubility to the molecule (e.g., carboxylic acid, carboxylic ester, carboxamido, morpholino, piperazinyl, imidazolyl, thiomorpholino, or tetrazolyl groups; both unsubstituted and substituted).

The terms "halo" and "halogen" refer to any radical of fluorine, chlorine, bromine or iodine. The terms "ring" and "ring system" refer to a ring comprising the delineated number of atoms, said atoms being carbon or, where indicated, a heteroatom such as nitrogen, oxygen or sulfur. The ring itself, as well as any substituents thereon, may be attached at any atom that allows a stable compound to be formed.

The term "aryl" refers to a 6-carbon monocyclic or 10-carbon bicyclic aromatic ring system wherein 0, 1, 2 or 3 atoms of each ring may be substituted by a substituent. Examples of aryl groups include phenyl, naphthyl and the like.

The term "heteroaryl" refers to an aromatic 5-8 membered monocyclic, 8-12 membered bicyclic, or 11-14 membered tricyclic ring system comprising 1-3 heteroatoms if monocyclic, 1-6 heteroatoms if bicyclic, or 1-9 heteroatoms if tricyclic, said heteroatoms selected from O, N, or S, wherein 0, 1, 2 or 3 atoms of each ring may be substituted by a substituent. Examples of heteroaryl groups include pyridyl, furyl or furanyl, imidazolyl, benzimidazolyl, pyrimidinyl, thiophenyl or thienyl, quinolinyl, indolyl, thiazolyl, and the like.

The term "heterocyclyl" refers to a nonaromatic 5-8 membered monocyclic, 8-12 membered bicyclic, or 11-14 membered tricyclic ring system comprising 1-3 heteroatoms if monocyclic, 1-6 heteroatoms if bicyclic, or 1-9 heteroatoms if tricyclic, said heteroatoms selected from O, N, or S, wherein 0, 1, 2 or 3 atoms of each ring may be substituted by a substituent. Examples of heterocyclyl groups include piperizinyl, pyrrolidinyl, dioxanyl, morpholinyl, tetrahydrofuranyl, and the like.

Combinations of substituents and variables envisioned by this invention are only those that result in the formation of stable compounds. The term "stable", as used herein, refers to compounds which possess stability sufficient to allow manufacture and which maintains the integrity of the compound for a sufficient period of time to be useful for the purposes detailed herein (e.g., therapeutic or prophylactic administration to a subject or antiseptic, wound dressing impregnation, sterilizant, or disinfectant applications).

A "subject in need of pain relief" does not necessarily experience pain currently, and "pain relief" includes less than 100% reduction in pain. For example, the invention can be used to treat a mammal, including a human patient, a dog, a cat, or a horse, for neuropathic pain attributable to any cause, e.g., postherpetic neuralgia, phantom or amputation stump pain, diabetic neuropathy, acquired immune deficiency syndrome neuropathy, back pain, and visceral pain (e.g., chronic pancreatitis). By "neuropathic pain" is meant pain arising from injury to or disturbance of the peripheral nervous system.

The compound can be administered locally or systemically, e.g., via an implant (for slow release, for example) or by intravenous bolus injection or infusion. An "implant" is any

device residing in a tissue deeper than the skin, in which the device produces a regulated or continuous release of a compound. Such devices are well known in the art of drug delivery (see, e.g., U.S. Patent No. 6,013,853). For example, a compound of Formula II can be prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811. The amount of compound administered at one time can be about 1 ng to 4 mg/m^2 body surface area (e.g., 80 ng to 1 mg/m^2 body surface area), and the compound can be formulated in an aqueous solution that optionally contains pharmaceutically acceptable carriers. Other suitable dosages include about 1 to 1000 mg/kg body weight (e.g., about 10 to 500, or about 100 mg/kg body weight).

Treatment in accordance with the invention produces relief of pain in patients whose current pain is resistant to other methods of pain relief, such as using opioid drugs. The invention can also be used in anticipation of pain to prevent pain.

The invention further relates to methods of selectively inhibiting, treating, or preventing neuropathic pain selectively over acute nociceptive pain comprising identifying a subject in need of pain relief, and administering a compound (or composition) of any of the formulae delineated herein, including those of Formula II. In another aspect, the method involves selectively inhibiting, treating, or preventing neuropathic pain selectively over nociceptive pain comprising identifying a subject in need of pain relief, and administering a compound (or composition) of any of the formulae delineated herein. In one aspect of these methods, selectively refers to inhibiting neuropathic pain to a greater extent than nociceptive pain. In another aspect of these methods, selectively refers to inhibiting neuropathic pain at least 50% more (e.g., >100% more, >200% more, 500% more) than nociceptive pain as determined by standard pain models, including those delineated in Borsook et al., US

5,905,069 (and references cited therein), issued May 18, 1999, and Abdi et al., Anesth. Analg. 91, 955-999 (2000).

The invention also relates to methods of assessing, identifying, or validating genes (or polypeptides encoded by those genes) as receptors involved in pain mediation, including neuropathic pain treatment. The methods comprise contacting a compound of any of the formulae herein with a test polypeptide and measuring the binding affinity of the compound and test polypeptide. Those polypeptides (and the genes encoding them) having a greater affinity for the compound are candidates having a greater likelihood of being directly involved in pain mediation, particularly neuropathic pain mediation. As such, they would be interesting targets for research and development studies for new mechanisms of pain mediation and for targets of inhibition by ligands (e.g., peptide or small molecule drugs) for treatment or prevention of pain. The analysis of the binding affinity of the compounds and receptors can be performed using assays, methods, and techniques known in the drug screening/design, genomics, and medicinal chemistry arts, including labeling (radiolabel, fluorescence) studies for detection of compound, target, or other ligand mediating a known peptide-ligand interaction, or can involve an indirect read-out (e.g., measuring presence of a marker whose release or formation is dependent upon the binding interaction of the compound and test polypeptide).

The invention further relates to methods of assessing the efficacy of potential pain drugs (e.g., peptides, chemical entities, small molecules). Because the compounds of the formulae herein are effective in mediating neuropathic pain, they are also useful as "standards" by which potential new pain drugs can be assessed. Such methods comprise evaluating a test compound (e.g., potential pain drug) against a compound of any the formulae herein (i.e., a standard), including a compound of Formula II, by subjecting the test compound and the compound of any of the formulae herein to a subject or medium (e.g., patient, animal model, cell culture, in vitro assay) that provides measure or assessment of the effectiveness of the test compound and the compound of any of the formulae herein in mediating pain or modulating the mechanism of pain. The method can further comprise evaluating the results of the compound testing to assess the effectiveness of the test compound as a pain drug. The measuring or assessing of the effectiveness of the compounds

in these methods can be performed by any number of appropriate techniques and protocols known in the art and readily available.

The invention also relates to methods of identifying new genes, receptors, or peptides that are involved in mediation of pain. These methods involve use of the compounds of any of the formulae herein, including those of Formula II, to investigate metabolic effects induced by the compounds, including effects involved in pain mediation. Such methods comprise administering the compounds of any of the formulae herein to a subject or medium (e.g., patient, animal model, cell culture, in vitro assay, tissue), collecting tissue from the subject or medium (e.g., dorsal root ganglion, nerve tissue, spinal cord tissue, or central nervous system (CNS) tissue), and evaluating the tissue for effects (e.g., identifying and/or quantifying: induction, suppression, indirect responses, marker production) of known or novel genes, receptors, or peptides. The known or novel genes, receptors, or peptides are interesting targets for new drugs to mediate pain, and provide new information and novel targets for new methods of pain mediation, treatment, or prevention. The evaluation in these methods can be performed using any number of appropriate techniques and protocols known in the art and readily available for measuring, detecting, and identifying genes, receptors, or peptides.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although suitable methods and materials are described below, methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

Other features and advantages of the invention will be apparent from the following detailed description, and from the claims.

Brief Description of the Drawing

Fig. 1 is a bar graph of force in grams (high force correlates with high pain threshold) versus time of testing, for untreated rats and rats treated with SAN-Gly. Error bars represent one standard deviation.

Fig. 2 is a bar graph of force in grams (high force correlates with high pain threshold) versus time of testing, for untreated rats and rats treated with SAN-Gly at 100 mg/Kg, 200 mg/Kg, and 300 mg/Kg. Error bars represent one standard deviation.

Detailed Description

The invention relates to methods of decreasing or preventing pain by administering to a subject a water-soluble spicamycin derivative (Formula II) in an amount sufficient to decrease or prevent the pain. Accordingly, the compound of the present invention can be administered via any appropriate route, e.g. intravenously, intraarterially, topically, nasally, via inhalation into the lungs, intraperitoneally, intrapleurally, orally, subcutaneously, intramuscularly, sublingually, intraepidermally, vaginally, or rectally. The compound can be formulated as a solution, suspension, suppository, tablet, granules, powder, capsules, ointment, or cream. A variety of additives can be added to these formulations, such as a solvent (e.g., water or physiological saline), solubilizing agent (e.g., ethanol, Polysorbates, or Cremophor EL7®), agent for achieving isotonicity, preservative, antioxidizing agent, excipient (e.g., lactose, starch, crystalline cellulose, mannitol, maltose, calcium hydrogen phosphate, light silicic acid anhydride, or calcium carbonate), binder (e.g., starch, polyvinylpyrrolidone, hydroxypropyl cellulose, ethyl cellulose, carboxy methyl cellulose, or gum arabic), lubricant (e.g., magnesium stearate, talc, or hardened oils), or stabilizer (e.g., lactose, mannitol, maltose, polysorbates, macrogels, or polyoxyethylene hardened castor oils). If suitable, the following compounds can also be added: glycerin, dimethylacetamide, sodium lactate, a surfactant, or a basic substance such as sodium hydroxide, ethylenediamine, ethanolamine, sodium bicarbonate, arginine, meglumine, or trisaminomethane. As discussed above, organic solvents (e.g., ethanol) are not required for pharmaceutical formulations containing compounds of Formula II. However, the solubilizing agents and organic materials listed above can be used if a hydrophobic material (e.g., a second analgesic) is included in the formulation, or if the pharmacokinetic characteristic of the formulation is to be

modulated. Pharmaceutical preparations such as solutions, tablets, granules, or capsules can be formed with these components or the like.

The dose of the compound of the present invention is determined in consideration of the results of animal experiments and various conditions. For example, any candidate compound for pain relief can be tested in the animal models described in the example below. More specific doses obviously vary depending on the administration method, the condition of the subject such as age, body weight, sex, sensitivity, food eaten, dosage intervals, medicines administered in combination, and the source, seriousness, and degree of pain. The optimal dose and the administration frequency under a given condition must be determined by the appropriate dosage test of a medical specialist based on the aforementioned guide.

Water-soluble, non-toxic, or less toxic derivatives of spicamycin, such as SAN and SAN-Gly, can be prepared using methods known in the art. For example, general synthetic strategies are described in U.S. Patent Nos. 5,461,036 and 5,631,238. These strategies can be adapted to attach any R group containing one or two carbons onto a sugar group, as shown in Formula II. A specific semi-synthetic strategy for preparing SAN and SAN-Gly is described in Kamishohara et al., J. Antibiotics 46:1439-1446, 1993; Kamishohara et al., Oncology Res. 6:383-390, 1994; and U.S. Patent Nos. 5,461,036 and 5,631,238. Synthetic chemistry transformations and protecting group methodologies (protection and deprotection) useful in synthesizing the inhibitor compounds described herein are known in the art and include, for example, those such as described in R. Larock, *Comprehensive Organic Transformations*, VCH Publishers (1989); T.W. Greene and P.G.M. Wuts, *Protective Groups in Organic Synthesis*, 2d. Ed., John Wiley and Sons (1991); L. Fieser and M. Fieser, *Fieser and Fieser's Reagents for Organic Synthesis*, John Wiley and Sons (1994); and L. Paquette, ed., *Encyclopedia of Reagents for Organic Synthesis*, John Wiley and Sons (1995), and respective subsequent editions thereof.

The compounds of this invention include all salt forms thereof. Examples of such salts include those derived from pharmaceutically acceptable inorganic and organic acids and bases. Examples of suitable acid salts include acetate, adipate, alginate, aspartate, benzoate, butyrate, citrate, fumarate, glycolate, hemisulfate, heptanoate, hexanoate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxyethanesulfonate, lactate, maleate, malonate, methanesulfonate, nicotinate, nitrate, oxalate, palmoate, pectinate, persulfate, picrate,

pivalate, propionate, salicylate, succinate, sulfate, tartrate, thiocyanate, tosylate and undecanoate. Other acids, such as oxalic, while not in themselves pharmaceutically acceptable, may be employed in the preparation of salts useful as intermediates in obtaining the compounds of the invention and their pharmaceutically acceptable acid addition salts. Salts derived from appropriate bases include alkali metal (e.g., sodium, potassium), alkaline earth metal (e.g., magnesium), ammonium and N-(alkyl)$_4^+$ salts. Compounds of the formulae herein include those having quaternization of any basic nitrogen-containing group therein. The compounds of this invention may contain one or more asymmetric centers and thus occur as racemates and racemic mixtures, single enantiomers, individual diastereomers and diastereomeric mixtures. All such isomeric forms of these compounds are expressly included in the present invention.

Neuropathic pain is pain derived from a lesion or disorder of the peripheral nervous system (reviewed in Woolf, *Acta Neurochir.* 58:125-130, 1993, and Bennett, Neuropathic Pain. In: Textbook of Pain , P.D. Wall and R. Malzack, eds., 201-224, Churchill Livingstone, Edinburgh (1994)). Patients with neuropathic pain typically present with a characteristic set of sensory disorders independent of the cause, including a constant scalding or burning pain, a partial loss of sensitivity, tactile or cold allodynia, or hyperpathia to repeated stimulation. Peripheral neuropathic pain includes a number of diverse conditions, the commonest of which are trigeminal neuralgia, postherpetic neuralgia, painful diabetic neurophathy, and the reflex sympathetic dystrophies including causalgia, mononeuropathies, and peripheral nerve injury.

Nociceptive pain is pain caused by an injury or disease outside the nervous system. It is often an on-going dull ache or pressure, rather than the sharper, trauma-like pain more characteristic of neuropathic pain. Examples of nociceptive pain include pain from cancer or arthritis, sprains, bone fractures, burns, bumps, bruises. With acute pain, the severity of pain directly correlates to the level of tissue damage. This provides a protective reflex, such as the reflex to move one's hand immediately if upon touching a sharp or hot object. This type of pain is a symptom of injured or diseased tissue, so that when the underlying problem is cured the pain goes away. In chronic pain, the pain differs from acute pain as it does not serve a protective or other biological function. Rather, the nerves continue to send pain messages to the brain even though there is no continuing tissue damage.

Due to the differences in the mechanism and types of pain, it can be advantageous to selectively treat or prevent neuropathic pain selectively over nociceptive pain. In other instances, the pain or pain symptoms can be a combination of both neuropathic and nociceptive, in those instances treatment of both types of pain can be appropriate.

Few non-surgical alternatives exist for a patient with a disabling pain resistant to opioid drugs. The methods of this invention provide alternative water-soluble pain relievers to such patients. Increased water solubility can increase absorption into the systemic circulation when administered orally to a subject, thereby increasing bioavailability of a drug.

In addition, water-soluble derivatives of spicamycin, such as SAN-Gly, are less cytotoxic than water-insoluble derivatives of spicamycin, such as KRN5500 (Kamishohara et al., Oncology Res. 6:383-390, 1994). This implies that water-soluble derivatives of spicamycin are safer for patients receiving repetitive administrations, as may be required for treating chronic pain.

The invention also contemplates combination formulations containing a water-soluble derivative of spicamycin and a second analgesic or drug, such as an anti-inflammatory agent (e.g., aspirin, acetaminophen, ibuprofen, naproxen, diclofenac, celecoxib, NSAIDS, COX-1 inhibitors, COX-2 inhibitors, steroids, steroid derivatives, glucocorticoids).

Without further elaboration, it is believed that one skilled in the art can, based on the above disclosure and the description below, utilize the present invention to its fullest extent. The following example is to be construed as merely illustrative of how one skilled in the art can practice the invention, and is not limitative of the remainder of the disclosure in any way. All patents and publications cited in this disclosure are hereby incorporated by reference.

Example 1

To determine whether relatively water-soluble, less toxic spicamycin derivatives are useful for pain relief, the compound SAN-Gly was semi-synthetically prepared as described in U.S. Patent No. 5,631,238. SAN-Gly was then formulated in saline.

Male Sprague-Dawley rats (Charles River Laboratories) weighing 150-200 g were used to evaluate the analgesic properties of the SAN-Gly formulation. The animals were housed in groups of three in plastic cages with soft bedding and under a 12 hour light/dark

cycle. Food and water were available *ad libitum*. After one week of acclimatization to the laboratory conditions, all animals were tested to establish a baseline mechanical allodynia.

Since different animal models of pain can provide different experimental results, SAN-Gly was tested using two rat models. All experiments were performed in a single-blinded fashion, i.e., the experimenter was not aware of which rats received the control saline and which rats received the SAN-Gly formulation.

One rat model used was based on an experimentally produced segmental spinal nerve injury (Kim et al., Pain 50:355-363, 1992). One week after the acclimatization to laboratory conditions, baseline measurements were recorded and surgery was performed as described in Kim et al., supra. Rats were anesthetized with halothane in oxygen and placed in a prone position. A midline skin incision at L4-S2 was made, and paraspinal muscles were separated from the spinous processes in the L4-S2 region. The left L5 and L6 spinal nerves were identified and tightly ligated with 6-O silk thread. The wound was then closed. At the end of surgery, anesthesia was discontinued, and the animals were returned to their cages with food pellets and water *ad libitum*. The animals recovered from anesthesia within approximately 10 minutes.

The rats were allowed to recover from surgery for at least a week. Ten to twelve days after surgery, animals were tested for allodynia by monitoring hypersensitivity to pinprick with von Frey filaments (VFF). The animals were placed on a mesh floor and covered by a transparent plastic box open at the bottom. Calibrated VFF (3.61, 3.84, 4.08, 4.31, 4.56, 4.74, 4.93, and 5.16) were applied to the plantar skin of the left hindpaw using the up-down method and a 50% foot withdrawal (paw flinching) as the pain threshold. Each VFF was tested by inserting it from below the rat and through the mesh floor, and applying it to the second, third, and fourth digits of the foot until the filament just bent (Chaplan et al., J. Neurosci. Methods 53:55-63, 1994). A trial consisted of four repetitive VFF applications (at a frequency of one per 10-15 seconds). Rats were then separated into two groups, one receiving saline and the other receiving the SAN-Gly formulation (100 mg/kg body weight; single bolus in the tail vein). Both groups were then monitored for allodynia at various time points after surgery.

The other rat model was based on an experimentally induced chronic constriction nerve injury (Bennett et al., Pain 33:87-107, 1988). Peripheral mononeurophathy was

produced in rats as described in Bennett et al., supra. Surgery was conducted on rats under halothane anesthesia to expose the common sciatic nerve. Then loose constrictive ligatures were placed around the nerve, the wound closed, and the animals allowed to recover for at least a week. Animals were then tested for allodynia, separated into two groups, and retested as described above.

The results using both animal models were combined and summarized in Fig. 1. The single injection of SAN-Gly resulted in a 60% recovery towards baseline pain behavior, and this increased pain tolerance persisted for at least 7 days after surgery. Thus, SAN-Gly proved to be useful as an analgesic in two animal models of pain.

Example 2

Electrophysiological recordings were done on 650 μm thick transverse slices of lumbar spinal cord (from spared-nerve injury animals) with attached L4 dorsal root that were perfused with ice cold Krebs' solution ([in mM]: NaCl 117, KCl 3.6, $CaCl_2$ 2.5, $MgCl_2$ 1.2, NaH_2PO_4 1.2, $NaHCO_3$ 25 and glucose 11) saturated with 95% O_2 and 5% CO_2. To record primary afferent-evoked excitatory postsynaptic currents (EPSCs) from lamina II, whole cells were patch-clamped and then voltage-clamped at -70 mV. Orthodromicstimulation of L4 dorsal root was performed with a suction electrode attached to a constant current stimulator and graded intensity stimulation sufficient to recruit different afferent populations (Aβ, Aδ, and C-fibers) was used. Resistance of patch pipettes were 5-10 MW when filled with (in mM): Cs_2SO_4 110, $CaCl_2$ 0.5, $MgCl_2$ 2, EGTA 5, HEPES 5, TEA 5, ATP-Mg salt 5. Currents were amplified (Axopatch 200A), filtered at 2 kHz, digitized at 5 kHz and analyzed using pCLAMP 6 (Axon Instruments). SAN-Gly was dissolved in ice cold KREBs and bath-applied at two concentrations (10 and 100 mM). It was observed in three out of five cells that 100 mM SAN-Gly increased the latency and reduced the amplitude of EPSCs, consistent with the hypothesis that it might play a role in inhibiting synaptic transmission in the dorsal horn. This effect was reversed upon washout of the drug. In addition, as expected, the same concentration of SAN had no effect. This example demonstrates that SAN-Gly and its derivatives are useful in treating neuropathic pain.

Other Embodiments

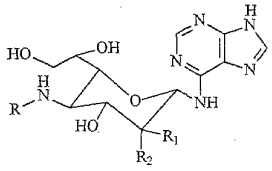
It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of this invention.

What is claimed is:

1. A method of providing pain relief, the method comprising

identifying a subject in need of pain relief; and

administering to the subject an amount of a compound of Formula II, or a salt thereof, effective to provide significant pain relief in the subject,



Formula II

wherein R_1 and R_2 are different from each other and represent —H or —OH, and R represents (1) a substituted or unsubstituted alkyl having one or two carbon atoms, or (2) —H.

2. The method of claim 1, wherein R is a substituted alkyl.

3. The method of claim 1, wherein R has two carbon atoms.

4. The method of claim 1, wherein R comprises a peptide bond.

5. The method of claim 1, wherein R comprises an amino group.

6. The method of claim 5, wherein the amino group is a primary amino group.

7. The method of claim 1, wherein R is —$COCH_2NH_2$.

8. The method of claim 7, wherein R_1 is —H and R_2 is —OH.

9. The method of claim 1, wherein R_1 is —H and R_2 is —OH.

10. The method of claim 1, wherein the pain is neuropathic pain.

11. The method of claim 10, wherein the pain is postherpetic neuralgia, phantom or amputation stump pain, diabetic neuropathy, acquired immune deficiency syndrome neuropathy, back pain, visceral pain, or chronic pancreatitic neuropathy.

12. The method of claim 1, wherein the pain is opioid-resistant.

13. The method of claim 1, wherein the subject is a mammal.

14. The method of claim 1, wherein the subject is a human.

15. The method of claim 1, wherein the compound is administered systemically.

16. The method of claim 1, wherein the compound is administered at a site of pain in the subject.

17. The method of claim 1, wherein the compound is administered via an implant.

18. The method of claim 17, wherein the implant provides slow release of the compound.

19. The method of claim 1, wherein the compound is administered intravenously.

20. The method of claim 1, wherein the amount administered is about 1 ng to 4 mg/m^2 body surface area.

21. The method of claim 1, wherein the amount administered is about 80 ng to 1 mg/m^2 patient body surface area.

22. The method of claim 1, wherein the amount administered is about 10 to 100 mg/kg body weight.

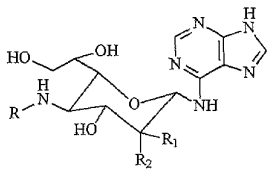
23. The method of claim 22, wherein the amount administered is about 100 mg/kg body weight.

24. The method of claim 1, wherein the compound is administered in an aqueous solution.

25. The method of claim 1 wherein neuropathic pain is selectively relieved over nociceptive pain.

26. A method for assessing a gene or test polypeptide encoded by the gene as a target for neuropathic pain treatment comprising:

contacting a compound of Formula II, or a salt thereof,



Formula II

wherein:

Each R group can independently be an H or alkyl group having 1 to 5 carbon atoms substituted with 1 to 3 independent R^3 or R^4;

Each R^3 is independently heterocyclyl or heteroaryl, either optionally substituted with 1-3 independent R^5;

Each R^4 is independently halogen, oxygen, sulfur, CF_3, SR^6, OR^6, $OC(O)R^6$, NR^6R^6, NR^6R^7, $COOR^6$, $C(O)R^6$, or $C(O)NR^6R^6$;

Each R^5 is independently C1-C10 alkyl; halo; haloalkyl; SR^6; OR^6; NR^6R^6; $COOR^6$; NO_2; CN; $C(O)R^6$; $C(O)NR^6R^6$; $OC(O)R^6$; $S(O)_2R^6$; $S(O)_2NR^6R^6$; $NR^6C(O)NR^6R^6$; $NR^6C(O)R^6$; $NR^6(COOR^6)$; $NR^6C(O)R^8$; $NR^6S(O)_2NR^6R^6$; $NR^6S(O)_2R^6$; $NR^6S(O)_2R^8$; or C1-C10 alkyl substituted with R^4 or R^8;

Each R^6 is independently H, C1-C10 alkyl; C2-C10 alkenyl; C2-C10 alkynyl; C3-C10 cycloalkyl; R^8; or C1-C10 alkyl substituted with R^8;

Each R^7 is independently $COOR^9$, $C(O)NR^9R^9$, $S(O)_2R^9$; or $S(O)_2NR^9R^9$;

Each R^8 is independently aryl, heteroaryl, or heterocyclyl; and

Each R^9 is independently H, C1-C10 alkyl, aryl, heteroaryl, or heterocyclyl;

with a test polypeptide; and

measuring the binding affinity of the compound of Formula II, or a salt thereof, and test polypeptide.

27. The method of claim 26, wherein R_1 and R_2 are different from each other and represent —H or —OH, and R represents (1) a substituted or unsubstituted alkyl having one or two carbon atoms, or (2) —H.

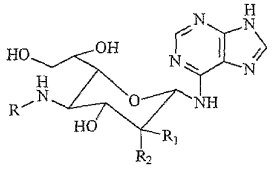
28. The method of claim 26, wherein:

Each R group can independently be an H or alkyl group having 1 to 2 carbon atoms substituted with 1 to 3 independent R^3 or R^4.

29. A method of providing pain relief, the method comprising

identifying a subject in need of pain relief; and

administering to the subject an amount of a compound of Formula II, or a salt thereof, effective to provide significant pain relief in the subject,



Formula II

wherein

Each R group can independently be an H or alkyl group having 1 to 5 carbon atoms substituted with 1 to 3 independent R^3 or R^4;

Each R^3 is independently heterocyclyl or heteroaryl, either optionally substituted with 1-3 independent R^5;

Each R^4 is independently halogen, oxygen, sulfur, CF_3, SR^6, OR^6, $OC(O)R^6$, NR^6R^6, NR^6R^7, $COOR^6$, $C(O)R^6$, or $C(O)NR^6R^6$;

Each R^5 is independently C1-C10 alkyl; halo; haloalkyl; SR^6; OR^6; NR^6R^6; $COOR^6$; NO_2; CN; $C(O)R^6$; $C(O)NR^6R^6$; $OC(O)R^6$; $S(O)_2R^6$; $S(O)_2NR^6R^6$; $NR^6C(O)NR^6R^6$; $NR^6C(O)R^6$; $NR^6(COOR^6)$; $NR^6C(O)R^8$; $NR^6S(O)_2NR^6R^6$; $NR^6S(O)_2R^6$; $NR^6S(O)_2R^8$; or C1-C10 alkyl substituted with R^4 or R^8;

Each R^6 is independently H, C1-C10 alkyl; C2-C10 alkenyl; C2-C10 alkynyl; C3-C10 cycloalkyl; R^8; or C1-C10 alkyl substituted with R^8;

Each R^7 is independently $COOR^9$, $C(O)NR^9R^9$, $S(O)_2R^9$; or $S(O)_2NR^9R^9$;

Each R^8 is independently aryl, heteroaryl, or heterocyclyl; and

Each R^9 is independently H, C1-C10 alkyl, aryl, heteroaryl, or heterocyclyl;

with a test polypeptide.

69

70 30. The method of claim 29, wherein the pain is neuropathic pain.

1 31. The method of claim 30, wherein the pain is postherpetic neuralgia, phantom or
2 amputation stump pain, diabetic neuropathy, acquired immune deficiency syndrome
3 neuropathy, back pain, visceral pain, or chronic pancreatitic neuropathy.

1 32. The method of claim 29, wherein the pain is opioid-resistant.

1 33. The method of claim 29, wherein the subject is a mammal.

1 34. The method of claim 29, wherein the subject is a human.

1 35. The method of claim 29, wherein the compound is administered systemically.

1 36. The method of claim 29, wherein the compound is administered at a site of pain
2 in the subject.

1 37. The method of claim 29, wherein the compound is administered via an implant.

1 38. The method of claim 37, wherein the implant provides slow release of the
2 compound.

1 39. The method of claim 29, wherein the compound is administered intravenously.

1 40. The method of claim 29, wherein the amount administered is about 1 ng to
2 4 mg/m^2 body surface area.

1 41. The method of claim 29, wherein the amount administered is about 80 ng to
2 1 mg/m^2 patient body surface area.

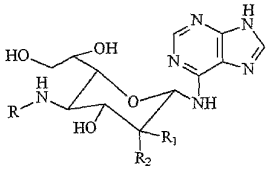
42. The method of claim 29, wherein the amount administered is about 10 to 100 mg/kg body weight.

43. The method of claim 42, wherein the amount administered is about 100 mg/kg body weight.

44. The method of claim 29, wherein the compound is administered in an aqueous solution.

45. The method of claim 29 wherein neuropathic pain is selectively relieved over nociceptive pain.

46. A method of identifying new genes, receptors, or peptides that are involved in mediation of pain comprising administering a compound of Formula II, or a salt thereof,



Formula II

wherein

Each R group can independently be an H or alkyl group having 1 to 5 carbon atoms substituted with 1 to 3 independent R^3 or R^4;

Each R^3 is independently heterocyclyl or heteroaryl, either optionally substituted with 1-3 independent R^5;

Each R^4 is independently halogen, oxygen, sulfur, CF_3, SR^6, OR^6, $OC(O)R^6$, NR^6R^6, NR^6R^7, $COOR^6$, $C(O)R^6$, or $C(O)NR^6R^6$;

Each R^5 is independently C1-C10 alkyl; halo; haloalkyl; SR^6; OR^6; NR^6R^6; $COOR^6$; NO_2; CN; $C(O)R^6$; $C(O)NR^6R^6$; $OC(O)R^6$; $S(O)_2R^6$; $S(O)_2NR^6R^6$; $NR^6C(O)NR^6R^6$; $NR^6C(O)R^6$; $NR^6(COOR^6)$; $NR^6C(O)R^8$; $NR^6S(O)_2NR^6R^6$; $NR^6S(O)_2R^6$; $NR^6S(O)_2R^8$; or C1-C10 alkyl substituted with R^4 or R^8;

Each R^6 is independently H, C1-C10 alkyl; C2-C10 alkenyl; C2-C10 alkynyl; C3-C10 cycloalkyl; R^8; or C1-C10 alkyl substituted with R^8;

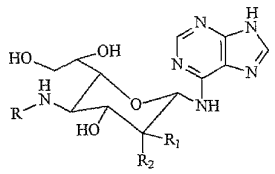
Each R^7 is independently $COOR^9$, $C(O)NR^9R^9$, $S(O)_2R^9$; or $S(O)_2NR^9R^9$;

Each R^8 is independently aryl, heteroaryl, or heterocyclyl; and

Each R^9 is independently H, C1-C10 alkyl, aryl, heteroaryl, or heterocyclyl;

to tissue, collecting the tissue, and evaluating the tissue for effects of known or novel genes, receptors, or peptides.

47. A method of assessing the efficacy of potential pain drugs comprising evaluating a test compound against a compound of *Formula II* , or salt thereof,



Formula II

wherein

Each R group can independently be an H or alkyl group having 1 to 5 carbon atoms substituted with 1 to 3 independent R^3 or R^4;

Each R^3 is independently heterocyclyl or heteroaryl, either optionally substituted with 1-3 independent R^5;

Each R^4 is independently halogen, oxygen, sulfur, CF_3, SR^6, OR^6, $OC(O)R^6$, NR^6R^6, NR^6R^7, $COOR^6$, $C(O)R^6$, or $C(O)NR^6R^6$;

Each R^5 is independently C1-C10 alkyl; halo; haloalkyl; SR^6; OR^6; NR^6R^6; $COOR^6$; NO_2; CN; $C(O)R^6$; $C(O)NR^6R^6$; $OC(O)R^6$; $S(O)_2R^6$; $S(O)_2NR^6R^6$; $NR^6C(O)NR^6R^6$; $NR^6C(O)R^6$; $NR^6(COOR^6)$; $NR^6C(O)R^8$; $NR^6S(O)_2NR^6R^6$; $NR^6S(O)_2R^6$; $NR^6S(O)_2R^8$; or C1-C10 alkyl substituted with R^4 or R^8;

Each R^6 is independently H, C1-C10 alkyl; C2-C10 alkenyl; C2-C10 alkynyl; C3-C10 cycloalkyl; R^8; or C1-C10 alkyl substituted with R^8;

Each R^7 is independently $COOR^9$, $C(O)NR^9R^9$, $S(O)_2R^9$; or $S(O)_2NR^9R^9$;

Each R^8 is independently aryl, heteroaryl, or heterocyclyl; and

Each R^9 is independently H, C1-C10 alkyl, aryl, heteroaryl, or heterocyclyl;

by subjecting the test compound and the compound Formula II to a subject or medium that provides a measure or assessment of the effectiveness of the test compound and the compound of Formula II.

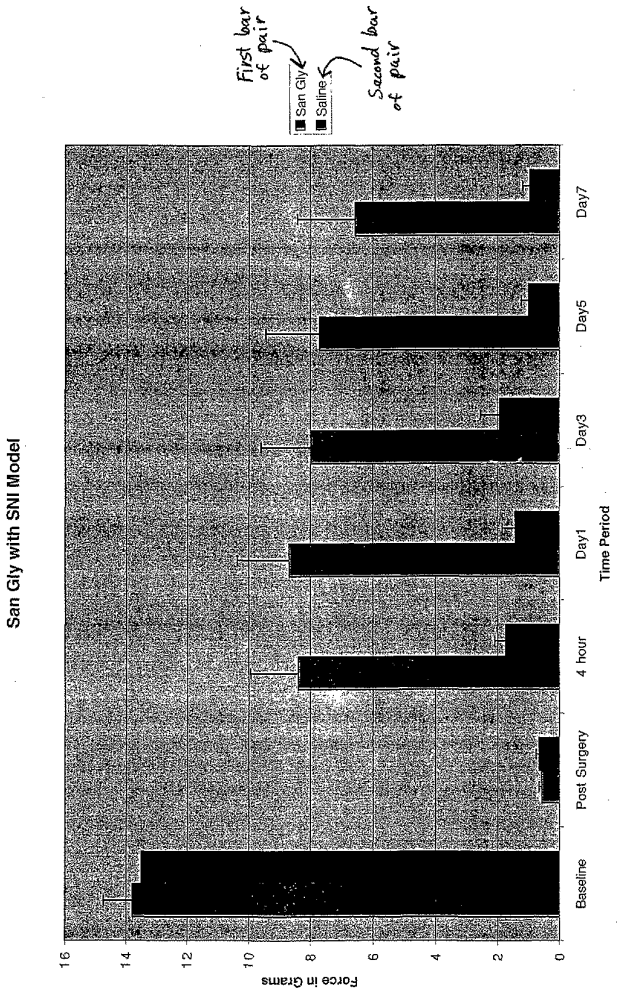

First bar of pair
Second bar of pair
San Gly
Saline
San Gly with SNI Model
Force in Grams
0
2
4
6
8
10
12
14
16
Baseline
Post Surgery
4 hour
Day1
Day3
Day5
Day7
Time Period

Fig. 1

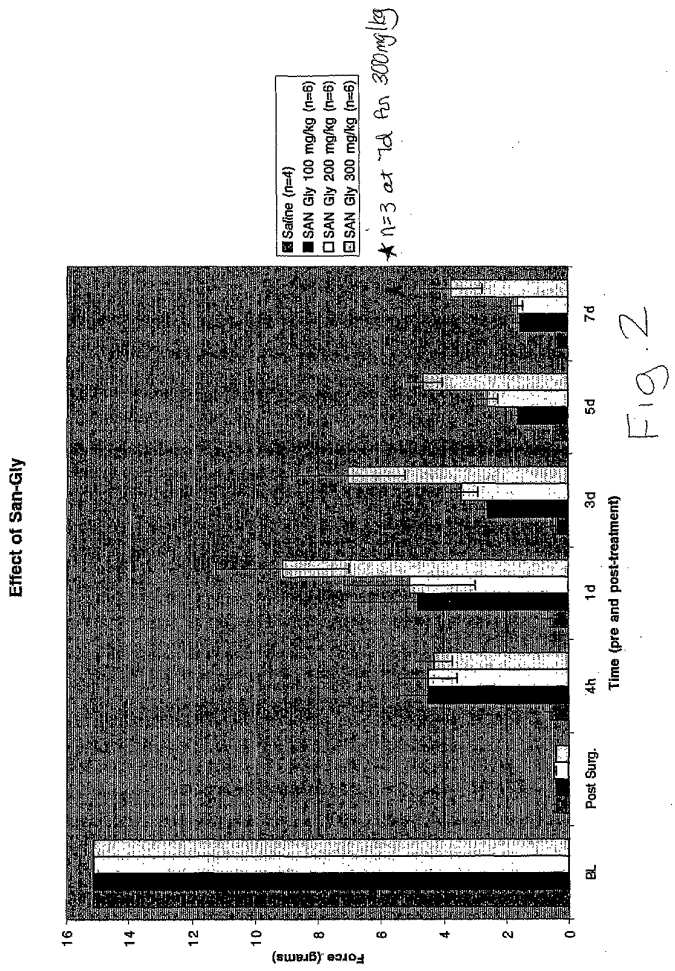

Effect of San-Gly
Saline (n=4)
SAN Gly 100 mg/kg (n=6)
SAN Gly 200 mg/kg (n=6)
SAN Gly 300 mg/kg (n=6)
★ n=3 at Td. for 300mg/kg
Force (grams)
0
2
4
6
8
10
12
14
16
BL
Post Surg.
4h
1d
3d
5d
7d
Time (pre and post-treatment)

Fig. 2

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(71) Applicant *(for all designated States except US)*: **THE GENERAL HOSPITAL CORPORATION** [US/US]; 55 Fruit Street, Boston, MA 02114-2696 (US).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: **BORSOOK, David** [GB/US]; 451 Strawberry Hill Road, Concord, MA 01742-5439 (US).

(74) Agent: **HSI, Jeffrey, D.**; Fish & Richardson P.C., 225 Franklin Street, Boston, MA 02110-8904 (US).

(81) Designated States *(national)*: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

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(54) Title: METHODS OF DECREASING OR PREVENTING PAIN USING SPICAMYCIN DERIVATIVES

(57) Abstract: Methods of providing pain relief by administering a water-soluble derivative of spicamycin. Methods of using pain mediation agents are also provided.

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(57) Abstract: Methods of providing pain relief by administering a water-soluble derivative of spicamycin. Methods of using pain mediation agents are also provided.

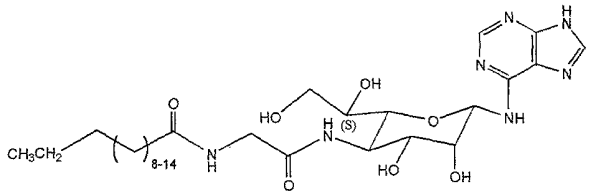
METHODS OF DECREASING OR PREVENTING PAIN USING SPICAMYCIN DERIVATIVES

Cross-Reference to Related Applications

This application claims the benefit of U.S. provisional application 60/234,382, filed September 20, 2000.

Background of the Invention

Spicamycin (SPM) is an antitumor antibiotic produced by the bacterium *Streptomyces*



alanosinicus 879-MT_3 (Hayakawa et al., Agric. Biol. Chem. 49:2685-2691, 1985). Spicamycin and its derivatives are also used for pain relief (U.S. Patent No. 5,905,069). Naturally occurring SPM has the following general structure, varying solely in the fatty acid moiety:

Formula I: Spicamycin

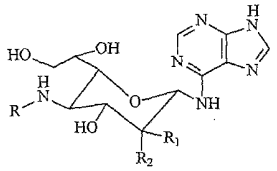
Synthetic variants of spicamycin and their use as an antitumor agent are described in Otake et al., U.S. Patent Nos. 5,461,036 and 5,631,238.

Summary of the Invention

The invention is based on the unexpected discovery that substantial removal of the fatty acid moiety in a subclass of spicamycin derivatives results in drugs especially useful for the treatment of pain. This subclass of spicamycin derivatives exhibit increased water-solubility relative to fatty acid-containing derivatives, and are therefore more amenable to

formulations suitable for human administration (e.g., formulations containing an aqueous physiological buffer). In addition, the potential toxicity of pharmaceutical formulations containing this subclass of spicamycin derivatives can be reduced because no toxic lipophilic carriers are needed in order to solubilize the active ingredients.

Thus, the invention features a method of providing pain relief by identifying a subject (e.g., a mammal, such as a human, dog, cat, or horse) in need of pain relief; and administering to the subject an amount of a compound of Formula II effective to provide significant pain relief in the subject.



Formula II

R_1 and R_2 are different from each other and represent —H or —OH, and R represents (1) a substituted or unsubstituted alkyl having one or two carbon atoms (i.e., no more than two carbon atoms), or (2) —H. For example, R can contain an amino group (e.g., a primary amino group), a carbonyl group or both. When R is $—COCH_2NH_2$, R_1 is —H, and R_2 is —OH, the compound is known as 4'-N-glycyl spicamycin amino nucleoside (SAN-Gly). When R is —H, the compound is known as SAN. See, e.g., Kamishohara et al., Oncology Res. 6:383-390, 1994. A salt of the compound of Formula II can also be used in the methods of the invention.

The compounds (and salts thereof) of Formula II useful in the methods herein also are those where R_1 and R_2 are independently H or OH, wherein R_1 and R_2 are not simultaneously the same and:

Each R group can independently be an H or alkyl group having 1 to 5 (e.g., 1 to 2, 1 to 3, 1 to 4) carbon atoms substituted with 1 to 3 independent R^3 or R^4;

Each R^3 is independently heterocyclyl or heteroaryl, either optionally substituted with 1-3 independent R^5;

Each R^4 is independently halogen, oxygen, sulfur, CF_3, SR^6, OR^6, $OC(O)R^6$, NR^6R^6, NR^6R^7, $COOR^6$, $C(O)R^6$, or $C(O)NR^6R^6$;

Each R^5 is independently C1-C10 alkyl; halo; haloalkyl; SR^6; OR^6; NR^6R^6; $COOR^6$; NO_2; CN; $C(O)R^6$; $C(O)NR^6R^6$; $OC(O)R^6$; $S(O)_2R^6$; $S(O)_2NR^6R^6$; $NR^6C(O)NR^6R^6$; $NR^6C(O)R^6$; $NR^6(COOR^6)$; $NR^6C(O)R^8$; $NR^6S(O)_2NR^6R^6$; $NR^6S(O)_2R^6$; $NR^6S(O)_2R^8$; or C1-C10 alkyl substituted with R^4 or R^8;

Each R^6 is independently H, C1-C10 alkyl; C2-C10 alkenyl; C2-C10 alkynyl; C3-C10 cycloalkyl; R^8; or C1-C10 alkyl substituted with R^8;

Each R^7 is independently $COOR^9$, $C(O)NR^9R^9$, $S(O)_2R^9$; or $S(O)_2NR^9R^9$;

Each R^8 is independently aryl, heteroaryl, or heterocyclyl;

Each R^9 is independently H, C1-C10 alkyl, aryl, heteroaryl, or heterocyclyl.

The term "alkyl" denotes a straight or branched hydrocarbon chain containing carbon atoms or cyclic hydrocarbon moieties. These alkyl groups may also contain one or more double bonds or triple bonds. By "substituted alkyl" is meant an alkyl in which an atom of the alkyl is substituted with, for example, a carbon, nitrogen, sulfur, oxygen, or halogen atom, or alternatively a nitrogen, sulfur, oxygen, or halogen atom.

Examples of substituents that can be attached to any atom of the alkyl group in a "substituted alkyl" include heterocyclyl groups; heteroaryl groups, amino groups, amido groups, alkoxy groups, acyloxy groups, thioalkoxy groups, acyl thioalkoxy groups, halogen groups, sulfonate groups, sulfonamide groups, ester groups, carboxylic acids, oxygen (e.g., a carbonyl group) and sulfur (e.g. a thiocarbonyl group). Substituents also include any chemical functional group that imparts improved water-solubility to the molecule (e.g., carboxylic acid, carboxylic ester, carboxamido, morpholino, piperazinyl, imidazolyl, thiomorpholino, or tetrazolyl groups; both unsubstituted and substituted).

The terms "halo" and "halogen" refer to any radical of fluorine, chlorine, bromine or iodine. The terms "ring" and "ring system" refer to a ring comprising the delineated number of atoms, said atoms being carbon or, where indicated, a heteroatom such as nitrogen, oxygen or sulfur. The ring itself, as well as any substituents thereon, may be attached at any atom that allows a stable compound to be formed.

The term "aryl" refers to a 6-carbon monocyclic or 10-carbon bicyclic aromatic ring system wherein 0, 1, 2 or 3 atoms of each ring may be substituted by a substituent. Examples of aryl groups include phenyl, naphthyl and the like.

The term "heteroaryl" refers to an aromatic 5-8 membered monocyclic, 8-12 membered bicyclic, or 11-14 membered tricyclic ring system comprising 1-3 heteroatoms if monocyclic, 1-6 heteroatoms if bicyclic, or 1-9 heteroatoms if tricyclic, said heteroatoms selected from O, N, or S, wherein 0, 1, 2 or 3 atoms of each ring may be substituted by a substituent. Examples of heteroaryl groups include pyridyl, furyl or furanyl, imidazolyl, benzimidazolyl, pyrimidinyl, thiophenyl or thienyl, quinolinyl, indolyl, thiazolyl, and the like.

The term "heterocyclyl" refers to a nonaromatic 5-8 membered monocyclic, 8-12 membered bicyclic, or 11-14 membered tricyclic ring system comprising 1-3 heteroatoms if monocyclic, 1-6 heteroatoms if bicyclic, or 1-9 heteroatoms if tricyclic, said heteroatoms selected from O, N, or S, wherein 0, 1, 2 or 3 atoms of each ring may be substituted by a substituent. Examples of heterocyclyl groups include piperizinyl, pyrrolidinyl, dioxanyl, morpholinyl, tetrahydrofuranyl, and the like.

Combinations of substituents and variables envisioned by this invention are only those that result in the formation of stable compounds. The term "stable", as used herein, refers to compounds which possess stability sufficient to allow manufacture and which maintains the integrity of the compound for a sufficient period of time to be useful for the purposes detailed herein (e.g., therapeutic or prophylactic administration to a subject or antiseptic, wound dressing impregnation, sterilizant, or disinfectant applications).

A "subject in need of pain relief" does not necessarily experience pain currently, and "pain relief" includes less than 100% reduction in pain. For example, the invention can be used to treat a mammal, including a human patient, a dog, a cat, or a horse, for neuropathic pain attributable to any cause, e.g., postherpetic neuralgia, phantom or amputation stump pain, diabetic neuropathy, acquired immune deficiency syndrome neuropathy, back pain, and visceral pain (e.g., chronic pancreatitis). By "neuropathic pain" is meant pain arising from injury to or disturbance of the peripheral nervous system.

The compound can be administered locally or systemically, e.g., via an implant (for slow release, for example) or by intravenous bolus injection or infusion. An "implant" is any

device residing in a tissue deeper than the skin, in which the device produces a regulated or continuous release of a compound. Such devices are well known in the art of drug delivery (see, e.g., U.S. Patent No. 6,013,853). For example, a compound of Formula II can be prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811. The amount of compound administered at one time can be about 1 ng to 4 mg/m^2 body surface area (e.g., 80 ng to 1 mg/m^2 body surface area), and the compound can be formulated in an aqueous solution that optionally contains pharmaceutically acceptable carriers. Other suitable dosages include about 1 to 1000 mg/kg body weight (e.g., about 10 to 500, or about 100 mg/kg body weight).

Treatment in accordance with the invention produces relief of pain in patients whose current pain is resistant to other methods of pain relief, such as using opioid drugs. The invention can also be used in anticipation of pain to prevent pain.

The invention further relates to methods of selectively inhibiting, treating, or preventing neuropathic pain selectively over acute nociceptive pain comprising identifying a subject in need of pain relief, and administering a compound (or composition) of any of the formulae delineated herein, including those of Formula II. In another aspect, the method involves selectively inhibiting, treating, or preventing neuropathic pain selectively over nociceptive pain comprising identifying a subject in need of pain relief, and administering a compound (or composition) of any of the formulae delineated herein. In one aspect of these methods, selectively refers to inhibiting neuropathic pain to a greater extent than nociceptive pain. In another aspect of these methods, selectively refers to inhibiting neuropathic pain at least 50% more (e.g., >100% more, >200% more, 500% more) than nociceptive pain as determined by standard pain models, including those delineated in Borsook et al., US

5,905,069 (and references cited therein), issued May 18, 1999, and Abdi et al., Anesth. Analg. 91, 955-999 (2000).

The invention also relates to methods of assessing, identifying, or validating genes (or polypeptides encoded by those genes) as receptors involved in pain mediation, including neuropathic pain treatment. The methods comprise contacting a compound of any of the formulae herein with a test polypeptide and measuring the binding affinity of the compound and test polypeptide. Those polypeptides (and the genes encoding them) having a greater affinity for the compound are candidates having a greater likelihood of being directly involved in pain mediation, particularly neuropathic pain mediation. As such, they would be interesting targets for research and development studies for new mechanisms of pain mediation and for targets of inhibition by ligands (e.g., peptide or small molecule drugs) for treatment or prevention of pain. The analysis of the binding affinity of the compounds and receptors can be performed using assays, methods, and techniques known in the drug screening/design, genomics, and medicinal chemistry arts, including labeling (radiolabel, fluorescence) studies for detection of compound, target, or other ligand mediating a known peptide-ligand interaction, or can involve an indirect read-out (e.g., measuring presence of a marker whose release or formation is dependent upon the binding interaction of the compound and test polypeptide).

The invention further relates to methods of assessing the efficacy of potential pain drugs (e.g., peptides, chemical entities, small molecules). Because the compounds of the formulae herein are effective in mediating neuropathic pain, they are also useful as "standards" by which potential new pain drugs can be assessed. Such methods comprise evaluating a test compound (e.g., potential pain drug) against a compound of any the formulae herein (i.e., a standard), including a compound of Formula II, by subjecting the test compound and the compound of any of the formulae herein to a subject or medium (e.g., patient, animal model, cell culture, in vitro assay) that provides measure or assessment of the effectiveness of the test compound and the compound of any of the formulae herein in mediating pain or modulating the mechanism of pain. The method can further comprise evaluating the results of the compound testing to assess the effectiveness of the test compound as a pain drug. The measuring or assessing of the effectiveness of the compounds

in these methods can be performed by any number of appropriate techniques and protocols known in the art and readily available.

The invention also relates to methods of identifying new genes, receptors, or peptides that are involved in mediation of pain. These methods involve use of the compounds of any of the formulae herein, including those of Formula II, to investigate metabolic effects induced by the compounds, including effects involved in pain mediation. Such methods comprise administering the compounds of any of the formulae herein to a subject or medium (e.g., patient, animal model, cell culture, in vitro assay, tissue), collecting tissue from the subject or medium (e.g., dorsal root ganglion, nerve tissue, spinal cord tissue, or central nervous system (CNS) tissue), and evaluating the tissue for effects (e.g., identifying and/or quantifying: induction, suppression, indirect responses, marker production) of known or novel genes, receptors, or peptides. The known or novel genes, receptors, or peptides are interesting targets for new drugs to mediate pain, and provide new information and novel targets for new methods of pain mediation, treatment, or prevention. The evaluation in these methods can be performed using any number of appropriate techniques and protocols known in the art and readily available for measuring, detecting, and identifying genes, receptors, or peptides.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although suitable methods and materials are described below, methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

Other features and advantages of the invention will be apparent from the following detailed description, and from the claims.

Brief Description of the Drawing

Fig. 1 is a bar graph of force in grams (high force correlates with high pain threshold) versus time of testing, for untreated rats and rats treated with SAN-Gly. Error bars represent one standard deviation.

Fig. 2 is a bar graph of force in grams (high force correlates with high pain threshold) versus time of testing, for untreated rats and rats treated with SAN-Gly at 100 mg/Kg, 200 mg/Kg, and 300 mg/Kg. Error bars represent one standard deviation.

Detailed Description

The invention relates to methods of decreasing or preventing pain by administering to a subject a water-soluble spicamycin derivative (Formula II) in an amount sufficient to decrease or prevent the pain. Accordingly, the compound of the present invention can be administered via any appropriate route, e.g. intravenously, intraarterially, topically, nasally, via inhalation into the lungs, intraperitoneally, intrapleurally, orally, subcutaneously, intramuscularly, sublingually, intraepidermally, vaginally, or rectally. The compound can be formulated as a solution, suspension, suppository, tablet, granules, powder, capsules, ointment, or cream. A variety of additives can be added to these formulations, such as a solvent (e.g., water or physiological saline), solubilizing agent (e.g., ethanol, Polysorbates, or Cremophor EL7®), agent for achieving isotonicity, preservative, antioxidizing agent, excipient (e.g., lactose, starch, crystalline cellulose, mannitol, maltose, calcium hydrogen phosphate, light silicic acid anhydride, or calcium carbonate), binder (e.g., starch, polyvinylpyrrolidone, hydroxypropyl cellulose, ethyl cellulose, carboxy methyl cellulose, or gum arabic), lubricant (e.g., magnesium stearate, talc, or hardened oils), or stabilizer (e.g., lactose, mannitol, maltose, polysorbates, macrogels, or polyoxyethylene hardened castor oils). If suitable, the following compounds can also be added: glycerin, dimethylacetamide, sodium lactate, a surfactant, or a basic substance such as sodium hydroxide, ethylenediamine, ethanolamine, sodium bicarbonate, arginine, meglumine, or trisaminomethane. As discussed above, organic solvents (e.g., ethanol) are not required for pharmaceutical formulations containing compounds of Formula II. However, the solubilizing agents and organic materials listed above can be used if a hydrophobic material (e.g., a second analgesic) is included in the formulation, or if the pharmacokinetic characteristic of the formulation is to be

modulated. Pharmaceutical preparations such as solutions, tablets, granules, or capsules can be formed with these components or the like.

The dose of the compound of the present invention is determined in consideration of the results of animal experiments and various conditions. For example, any candidate compound for pain relief can be tested in the animal models described in the example below. More specific doses obviously vary depending on the administration method, the condition of the subject such as age, body weight, sex, sensitivity, food eaten, dosage intervals, medicines administered in combination, and the source, seriousness, and degree of pain. The optimal dose and the administration frequency under a given condition must be determined by the appropriate dosage test of a medical specialist based on the aforementioned guide.

Water-soluble, non-toxic, or less toxic derivatives of spicamycin, such as SAN and SAN-Gly, can be prepared using methods known in the art. For example, general synthetic strategies are described in U.S. Patent Nos. 5,461,036 and 5,631,238. These strategies can be adapted to attach any R group containing one or two carbons onto a sugar group, as shown in Formula II. A specific semi-synthetic strategy for preparing SAN and SAN-Gly is described in Kamishohara et al., J. Antibiotics 46:1439-1446, 1993; Kamishohara et al., Oncology Res. 6:383-390, 1994; and U.S. Patent Nos. 5,461,036 and 5,631,238. Synthetic chemistry transformations and protecting group methodologies (protection and deprotection) useful in synthesizing the inhibitor compounds described herein are known in the art and include, for example, those such as described in R. Larock, *Comprehensive Organic Transformations*, VCH Publishers (1989); T.W. Greene and P.G.M. Wuts, *Protective Groups in Organic Synthesis*, 2d. Ed., John Wiley and Sons (1991); L. Fieser and M. Fieser, *Fieser and Fieser's Reagents for Organic Synthesis*, John Wiley and Sons (1994); and L. Paquette, ed., *Encyclopedia of Reagents for Organic Synthesis*, John Wiley and Sons (1995), and respective subsequent editions thereof.

The compounds of this invention include all salt forms thereof. Examples of such salts include those derived from pharmaceutically acceptable inorganic and organic acids and bases. Examples of suitable acid salts include acetate, adipate, alginate, aspartate, benzoate, butyrate, citrate, fumarate, glycolate, hemisulfate, heptanoate, hexanoate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxyethanesulfonate, lactate, maleate, malonate, methanesulfonate, nicotinate, nitrate, oxalate, palmoate, pectinate, persulfate, picrate,

pivalate, propionate, salicylate, succinate, sulfate, tartrate, thiocyanate, tosylate and undecanoate. Other acids, such as oxalic, while not in themselves pharmaceutically acceptable, may be employed in the preparation of salts useful as intermediates in obtaining the compounds of the invention and their pharmaceutically acceptable acid addition salts. Salts derived from appropriate bases include alkali metal (e.g., sodium, potassium), alkaline earth metal (e.g., magnesium), ammonium and N-(alkyl)$_4^+$ salts. Compounds of the formulae herein include those having quaternization of any basic nitrogen-containing group therein. The compounds of this invention may contain one or more asymmetric centers and thus occur as racemates and racemic mixtures, single enantiomers, individual diastereomers and diastereomeric mixtures. All such isomeric forms of these compounds are expressly included in the present invention.

Neuropathic pain is pain derived from a lesion or disorder of the peripheral nervous system (reviewed in Woolf, *Acta Neurochir*. 58:125-130, 1993, and Bennett, Neuropathic Pain. In: Textbook of Pain , P.D. Wall and R. Malzack, eds., 201-224, Churchill Livingstone, Edinburgh (1994)). Patients with neuropathic pain typically present with a characteristic set of sensory disorders independent of the cause, including a constant scalding or burning pain, a partial loss of sensitivity, tactile or cold allodynia, or hyperpathia to repeated stimulation. Peripheral neuropathic pain includes a number of diverse conditions, the commonest of which are trigeminal neuralgia, postherpetic neuralgia, painful diabetic neurophathy, and the reflex sympathetic dystrophies including causalgia, mononeuropathies, and peripheral nerve injury.

Nociceptive pain is pain caused by an injury or disease outside the nervous system. It is often an on-going dull ache or pressure, rather than the sharper, trauma-like pain more characteristic of neuropathic pain. Examples of nociceptive pain include pain from cancer or arthritis, sprains, bone fractures, burns, bumps, bruises. With acute pain, the severity of pain directly correlates to the level of tissue damage. This provides a protective reflex, such as the reflex to move one's hand immediately if upon touching a sharp or hot object. This type of pain is a symptom of injured or diseased tissue, so that when the underlying problem is cured the pain goes away. In chronic pain, the pain differs from acute pain as it does not serve a protective or other biological function. Rather, the nerves continue to send pain messages to the brain even though there is no continuing tissue damage.

Due to the differences in the mechanism and types of pain, it can be advantageous to selectively treat or prevent neuropathic pain selectively over nociceptive pain. In other instances, the pain or pain symptoms can be a combination of both neuropathic and nociceptive, in those instances treatment of both types of pain can be appropriate.

Few non-surgical alternatives exist for a patient with a disabling pain resistant to opioid drugs. The methods of this invention provide alternative water-soluble pain relievers to such patients. Increased water solubility can increase absorption into the systemic circulation when administered orally to a subject, thereby increasing bioavailability of a drug.

In addition, water-soluble derivatives of spicamycin, such as SAN-Gly, are less cytotoxic than water-insoluble derivatives of spicamycin, such as KRN5500 (Kamishohara et al., Oncology Res. 6:383-390, 1994). This implies that water-soluble derivatives of spicamycin are safer for patients receiving repetitive administrations, as may be required for treating chronic pain.

The invention also contemplates combination formulations containing a water-soluble derivative of spicamycin and a second analgesic or drug, such as an anti-inflammatory agent (e.g., aspirin, acetaminophen, ibuprofen, naproxen, diclofenac, celecoxib, NSAIDS, COX-1 inhibitors, COX-2 inhibitors, steroids, steroid derivatives, glucocorticoids).

Without further elaboration, it is believed that one skilled in the art can, based on the above disclosure and the description below, utilize the present invention to its fullest extent. The following example is to be construed as merely illustrative of how one skilled in the art can practice the invention, and is not limitative of the remainder of the disclosure in any way. All patents and publications cited in this disclosure are hereby incorporated by reference.

Example 1

To determine whether relatively water-soluble, less toxic spicamycin derivatives are useful for pain relief, the compound SAN-Gly was semi-synthetically prepared as described in U.S. Patent No. 5,631,238. SAN-Gly was then formulated in saline.

Male Sprague-Dawley rats (Charles River Laboratories) weighing 150-200 g were used to evaluate the analgesic properties of the SAN-Gly formulation. The animals were housed in groups of three in plastic cages with soft bedding and under a 12 hour light/dark

cycle. Food and water were available *ad libitum*. After one week of acclimatization to the laboratory conditions, all animals were tested to establish a baseline mechanical allodynia.

Since different animal models of pain can provide different experimental results, SAN-Gly was tested using two rat models. All experiments were performed in a single-blinded fashion, i.e., the experimenter was not aware of which rats received the control saline and which rats received the SAN-Gly formulation.

One rat model used was based on an experimentally produced segmental spinal nerve injury (Kim et al., Pain 50:355-363, 1992). One week after the acclimatization to laboratory conditions, baseline measurements were recorded and surgery was performed as described in Kim et al., supra. Rats were anesthetized with halothane in oxygen and placed in a prone position. A midline skin incision at L4-S2 was made, and paraspinal muscles were separated from the spinous processes in the L4-S2 region. The left L5 and L6 spinal nerves were identified and tightly ligated with 6-O silk thread. The wound was then closed. At the end of surgery, anesthesia was discontinued, and the animals were returned to their cages with food pellets and water *ad libitum*. The animals recovered from anesthesia within approximately 10 minutes.

The rats were allowed to recover from surgery for at least a week. Ten to twelve days after surgery, animals were tested for allodynia by monitoring hypersensitivity to pinprick with von Frey filaments (VFF). The animals were placed on a mesh floor and covered by a transparent plastic box open at the bottom. Calibrated VFF (3.61, 3.84, 4.08, 4.31, 4.56, 4.74, 4.93, and 5.16) were applied to the plantar skin of the left hindpaw using the up-down method and a 50% foot withdrawal (paw flinching) as the pain threshold. Each VFF was tested by inserting it from below the rat and through the mesh floor, and applying it to the second, third, and fourth digits of the foot until the filament just bent (Chaplan et al., J. Neurosci. Methods 53:55-63, 1994). A trial consisted of four repetitive VFF applications (at a frequency of one per 10-15 seconds). Rats were then separated into two groups, one receiving saline and the other receiving the SAN-Gly formulation (100 mg/kg body weight; single bolus in the tail vein). Both groups were then monitored for allodynia at various time points after surgery.

The other rat model was based on an experimentally induced chronic constriction nerve injury (Bennett et al., Pain 33:87-107, 1988). Peripheral mononeurophathy was

produced in rats as described in Bennett et al., supra. Surgery was conducted on rats under halothane anesthesia to expose the common sciatic nerve. Then loose constrictive ligatures were placed around the nerve, the wound closed, and the animals allowed to recover for at least a week. Animals were then tested for allodynia, separated into two groups, and retested as described above.

The results using both animal models were combined and summarized in Fig. 1. The single injection of SAN-Gly resulted in a 60% recovery towards baseline pain behavior, and this increased pain tolerance persisted for at least 7 days after surgery. Thus, SAN-Gly proved to be useful as an analgesic in two animal models of pain.

Example 2

Electrophysiological recordings were done on 650 μm thick transverse slices of lumbar spinal cord (from spared-nerve injury animals) with attached L4 dorsal root that were perfused with ice cold Krebs' solution ([in mM]: NaCl 117, KCl 3.6, $CaCl_2$ 2.5, $MgCl_2$ 1.2, NaH_2PO_4 1.2, $NaHCO_3$ 25 and glucose 11) saturated with 95% O_2 and 5% CO_2. To record primary afferent-evoked excitatory postsynaptic currents (EPSCs) from lamina II, whole cells were patch-clamped and then voltage-clamped at -70 mV. Orthodromicstimulation of L4 dorsal root was performed with a suction electrode attached to a constant current stimulator and graded intensity stimulation sufficient to recruit different afferent populations (Aβ, Aδ, and C-fibers) was used. Resistance of patch pipettes were 5-10 MW when filled with (in mM): Cs_2SO_4 110, $CaCl_2$ 0.5, $MgCl_2$ 2, EGTA 5, HEPES 5, TEA 5, ATP-Mg salt 5. Currents were amplified (Axopatch 200A), filtered at 2 kHz, digitized at 5 kHz and analyzed using pCLAMP 6 (Axon Instruments). SAN-Gly was dissolved in ice cold KREBs and bath-applied at two concentrations (10 and 100 mM). It was observed in three out of five cells that 100 mM SAN-Gly increased the latency and reduced the amplitude of EPSCs, consistent with the hypothesis that it might play a role in inhibiting synaptic transmission in the dorsal horn. This effect was reversed upon washout of the drug. In addition, as expected, the same concentration of SAN had no effect. This example demonstrates that SAN-Gly and its derivatives are useful in treating neuropathic pain.

Other Embodiments

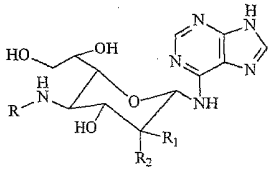
It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of this invention.

What is claimed is:

1. A method of providing pain relief, the method comprising

identifying a subject in need of pain relief; and

administering to the subject an amount of a compound of Formula II, or a salt thereof, effective to provide significant pain relief in the subject,



Formula II

wherein R_1 and R_2 are different from each other and represent —H or —OH, and R represents (1) a substituted or unsubstituted alkyl having one or two carbon atoms, or (2) —H.

2. The method of claim 1, wherein R is a substituted alkyl.

3. The method of claim 1, wherein R has two carbon atoms.

4. The method of claim 1, wherein R comprises a peptide bond.

5. The method of claim 1, wherein R comprises an amino group.

6. The method of claim 5, wherein the amino group is a primary amino group.

7. The method of claim 1, wherein R is —$COCH_2NH_2$.

8. The method of claim 7, wherein R_1 is —H and R_2 is —OH.

9. The method of claim 1, wherein R_1 is —H and R_2 is —OH.

10. The method of claim 1, wherein the pain is neuropathic pain.

11. The method of claim 10, wherein the pain is postherpetic neuralgia, phantom or amputation stump pain, diabetic neuropathy, acquired immune deficiency syndrome neuropathy, back pain, visceral pain, or chronic pancreatitic neuropathy.

12. The method of claim 1, wherein the pain is opioid-resistant.

13. The method of claim 1, wherein the subject is a mammal.

14. The method of claim 1, wherein the subject is a human.

15. The method of claim 1, wherein the compound is administered systemically.

16. The method of claim 1, wherein the compound is administered at a site of pain in the subject.

17. The method of claim 1, wherein the compound is administered via an implant.

18. The method of claim 17, wherein the implant provides slow release of the compound.

19. The method of claim 1, wherein the compound is administered intravenously.

20. The method of claim 1, wherein the amount administered is about 1 ng to 4 mg/m^2 body surface area.

21. The method of claim 1, wherein the amount administered is about 80 ng to 1 mg/m^2 patient body surface area.

22. The method of claim 1, wherein the amount administered is about 10 to 100 mg/kg body weight.

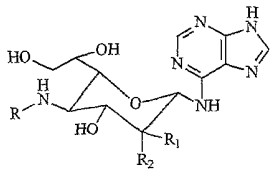
23. The method of claim 22, wherein the amount administered is about 100 mg/kg body weight.

24. The method of claim 1, wherein the compound is administered in an aqueous solution.

25. The method of claim 1 wherein neuropathic pain is selectively relieved over nociceptive pain.

26. A method for assessing a gene or test polypeptide encoded by the gene as a target for neuropathic pain treatment comprising:

contacting a compound of Formula II, or a salt thereof,



Formula II

wherein:

Each R group can independently be an H or alkyl group having 1 to 5 carbon atoms substituted with 1 to 3 independent R^3 or R^4;

Each R^3 is independently heterocyclyl or heteroaryl, either optionally substituted with 1-3 independent R^5;

Each R^4 is independently halogen, oxygen, sulfur, CF_3, SR^6, OR^6, $OC(O)R^6$, NR^6R^6, NR^6R^7, $COOR^6$, $C(O)R^6$, or $C(O)NR^6R^6$;

Each R^5 is independently C1-C10 alkyl; halo; haloalkyl; SR^6; OR^6; NR^6R^6; $COOR^6$; NO_2; CN; $C(O)R^6$; $C(O)NR^6R^6$; $OC(O)R^6$; $S(O)_2R^6$; $S(O)_2NR^6R^6$; $NR^6C(O)NR^6R^6$; $NR^6C(O)R^6$; $NR^6(COOR^6)$; $NR^6C(O)R^8$; $NR^6S(O)_2NR^6R^6$; $NR^6S(O)_2R^6$; $NR^6S(O)_2R^8$; or C1-C10 alkyl substituted with R^4 or R^8;

Each R^6 is independently H, C1-C10 alkyl; C2-C10 alkenyl; C2-C10 alkynyl; C3-C10 cycloalkyl; R^8; or C1-C10 alkyl substituted with R^8;

Each R^7 is independently $COOR^9$, $C(O)NR^9R^9$, $S(O)_2R^9$; or $S(O)_2NR^9R^9$;

Each R^8 is independently aryl, heteroaryl, or heterocyclyl; and

Each R^9 is independently H, C1-C10 alkyl, aryl, heteroaryl, or heterocyclyl;

with a test polypeptide; and

measuring the binding affinity of the compound of Formula II, or a salt thereof, and test polypeptide.

27. The method of claim 26, wherein R_1 and R_2 are different from each other and represent —H or —OH, and R represents (1) a substituted or unsubstituted alkyl having one or two carbon atoms, or (2) —H.

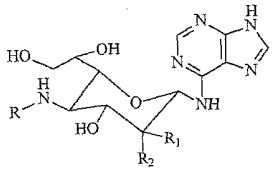
28. The method of claim 26, wherein:

Each R group can independently be an H or alkyl group having 1 to 2 carbon atoms substituted with 1 to 3 independent R^3 or R^4.

29. A method of providing pain relief, the method comprising

identifying a subject in need of pain relief; and

administering to the subject an amount of a compound of Formula II, or a salt thereof, effective to provide significant pain relief in the subject,



Formula II

wherein

Each R group can independently be an H or alkyl group having 1 to 5 carbon atoms substituted with 1 to 3 independent R^3 or R^4;

Each R^3 is independently heterocyclyl or heteroaryl, either optionally substituted with 1-3 independent R^5;

Each R^4 is independently halogen, oxygen, sulfur, CF_3, SR^6, OR^6, $OC(O)R^6$, NR^6R^6, NR^6R^7, $COOR^6$, $C(O)R^6$, or $C(O)NR^6R^6$;

Each R^5 is independently C1-C10 alkyl; halo; haloalkyl; SR^6; OR^6; NR^6R^6; $COOR^6$; NO_2; CN; $C(O)R^6$; $C(O)NR^6R^6$; $OC(O)R^6$; $S(O)_2R^6$; $S(O)_2NR^6R^6$; $NR^6C(O)NR^6R^6$; $NR^6C(O)R^6$; $NR^6(COOR^6)$; $NR^6C(O)R^8$; $NR^6S(O)_2NR^6R^6$; $NR^6S(O)_2R^6$; $NR^6S(O)_2R^8$; or C1-C10 alkyl substituted with R^4 or R^8;

Each R^6 is independently H, C1-C10 alkyl; C2-C10 alkenyl; C2-C10 alkynyl; C3-C10 cycloalkyl; R^8; or C1-C10 alkyl substituted with R^8;

Each R^7 is independently $COOR^9$, $C(O)NR^9R^9$, $S(O)_2R^9$; or $S(O)_2NR^9R^9$;

Each R^8 is independently aryl, heteroaryl, or heterocyclyl; and

Each R^9 is independently H, C1-C10 alkyl, aryl, heteroaryl, or heterocyclyl;

with a test polypeptide.

69

70 30. The method of claim 29, wherein the pain is neuropathic pain.

1 31. The method of claim 30, wherein the pain is postherpetic neuralgia, phantom or
2 amputation stump pain, diabetic neuropathy, acquired immune deficiency syndrome
3 neuropathy, back pain, visceral pain, or chronic pancreatitic neuropathy.

1 32. The method of claim 29, wherein the pain is opioid-resistant.

1 33. The method of claim 29, wherein the subject is a mammal.

1 34. The method of claim 29, wherein the subject is a human.

1 35. The method of claim 29, wherein the compound is administered systemically.

1 36. The method of claim 29, wherein the compound is administered at a site of pain
2 in the subject.

1 37. The method of claim 29, wherein the compound is administered via an implant.

1 38. The method of claim 37, wherein the implant provides slow release of the
2 compound.

1 39. The method of claim 29, wherein the compound is administered intravenously.

1 40. The method of claim 29, wherein the amount administered is about 1 ng to
2 4 mg/m^2 body surface area.

1 41. The method of claim 29, wherein the amount administered is about 80 ng to
2 1 mg/m^2 patient body surface area.

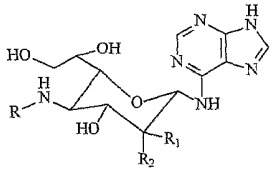
42. The method of claim 29, wherein the amount administered is about 10 to 100 mg/kg body weight.

43. The method of claim 42, wherein the amount administered is about 100 mg/kg body weight.

44. The method of claim 29, wherein the compound is administered in an aqueous solution.

45. The method of claim 29 wherein neuropathic pain is selectively relieved over nociceptive pain.

46. A method of identifying new genes, receptors, or peptides that are involved in mediation of pain comprising administering a compound of Formula II, or a salt thereof,



Formula II

wherein

Each R group can independently be an H or alkyl group having 1 to 5 carbon atoms substituted with 1 to 3 independent R^3 or R^4;

Each R^3 is independently heterocyclyl or heteroaryl, either optionally substituted with 1-3 independent R^5;

Each R^4 is independently halogen, oxygen, sulfur, CF_3, SR^6, OR^6, $OC(O)R^6$, NR^6R^6, NR^6R^7, $COOR^6$, $C(O)R^6$, or $C(O)NR^6R^6$;

Each R^5 is independently C1-C10 alkyl; halo; haloalkyl; SR^6; OR^6; NR^6R^6; $COOR^6$; NO_2; CN; $C(O)R^6$; $C(O)NR^6R^6$; $OC(O)R^6$; $S(O)_2R^6$; $S(O)_2NR^6R^6$; $NR^6C(O)NR^6R^6$; $NR^6C(O)R^6$; $NR^6(COOR^6)$; $NR^6C(O)R^8$; $NR^6S(O)_2NR^6R^6$; $NR^6S(O)_2R^6$; $NR^6S(O)_2R^8$; or C1-C10 alkyl substituted with R^4 or R^8;

Each R^6 is independently H, C1-C10 alkyl; C2-C10 alkenyl; C2-C10 alkynyl; C3-C10 cycloalkyl; R^8; or C1-C10 alkyl substituted with R^8;

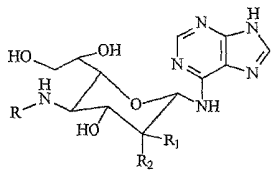
Each R^7 is independently $COOR^9$, $C(O)NR^9R^9$, $S(O)_2R^9$; or $S(O)_2NR^9R^9$;

Each R^8 is independently aryl, heteroaryl, or heterocyclyl; and

Each R^9 is independently H, C1-C10 alkyl, aryl, heteroaryl, or heterocyclyl;

to tissue, collecting the tissue, and evaluating the tissue for effects of known or novel genes, receptors, or peptides.

47. A method of assessing the efficacy of potential pain drugs comprising evaluating a test compound against a compound of Formula II , or salt thereof,



Formula II

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Each R group can independently be an H or alkyl group having 1 to 5 carbon atoms substituted with 1 to 3 independent R^3 or R^4;

Each R^3 is independently heterocyclyl or heteroaryl, either optionally substituted with 1-3 independent R^5;

Each R^4 is independently halogen, oxygen, sulfur, CF_3, SR^6, OR^6, $OC(O)R^6$, NR^6R^6, NR^6R^7, $COOR^6$, $C(O)R^6$, or $C(O)NR^6R^6$;

Each R^5 is independently C1-C10 alkyl; halo; haloalkyl; SR^6; OR^6; NR^6R^6; $COOR^6$; NO_2; CN; $C(O)R^6$; $C(O)NR^6R^6$; $OC(O)R^6$; $S(O)_2R^6$; $S(O)_2NR^6R^6$; $NR^6C(O)NR^6R^6$; $NR^6C(O)R^6$; $NR^6(COOR^6)$; $NR^6C(O)R^8$; $NR^6S(O)_2NR^6R^6$; $NR^6S(O)_2R^6$; $NR^6S(O)_2R^8$; or C1-C10 alkyl substituted with R^4 or R^8;

Each R^6 is independently H, C1-C10 alkyl; C2-C10 alkenyl; C2-C10 alkynyl; C3-C10 cycloalkyl; R^8; or C1-C10 alkyl substituted with R^8;

Each R^7 is independently $COOR^9$, $C(O)NR^9R^9$, $S(O)_2R^9$; or $S(O)_2NR^9R^9$;

Each R^8 is independently aryl, heteroaryl, or heterocyclyl; and

Each R^9 is independently H, C1-C10 alkyl, aryl, heteroaryl, or heterocyclyl;

by subjecting the test compound and the compound Formula II to a subject or medium that provides a measure or assessment of the effectiveness of the test compound and the compound of Formula II.

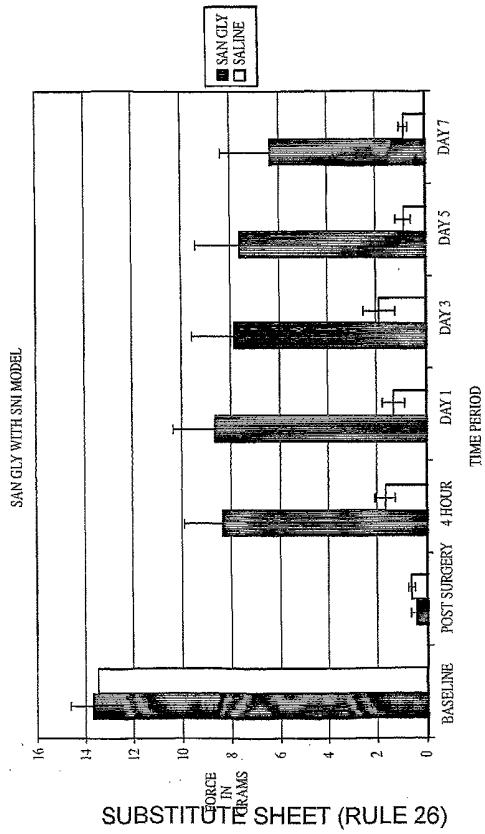
SAN GLY
SALINE
SAN GLY WITH SNI MODEL
FORCE IN GRAMS
16
14
12
10
8
6
4
2
0
BASELINE
POST SURGERY
4 HOUR
DAY 1
DAY 3
DAY 5
DAY 7
TIME PERIOD

FIG. 1

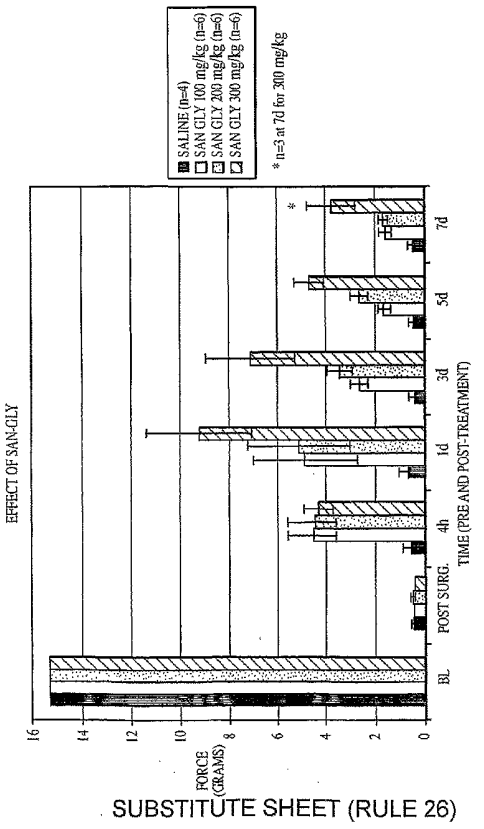
SALINE (n=4)
SAN GLY 100 mg/kg (n=6)
SAN GLY 200 mg/kg (n=6)
SAN GLY 300 mg/kg (n=6)
* n=3 at 7d for 300 mg/kg
EFFECT OF SAN-GLY
FORCE (GRAMS)
16
14
12
10
8
6
4
2
0
BL
POST SURG.
4h
1d
3d
5d
7d
TIME (PRE AND POST-TREATMENT)

FIG. 2

【国際調査報告】

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/29371

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) : A61K 31/52
US CL : 514/266
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 514/266

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
STN: embase, medline, biosis, caplus, uspatfull and Pub Med.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5,905,069 A(BORSOOK et al) 18 May 1999 (05.18.1999), whole document.	1-47
X, P	Database PubMed, US National Library of Medicine, (Bethesda, MD, USA), No. 11004056, The effects of KRN5500, a spicamycin derivative, on neuropathic and nociceptive pain models in rats, VILASSOVA et. al., October 2000.	1-47

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
01 November 2001 (01.11.2001)	30 DEC 2002
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703)305-3230	Authorized officer Minna Moezie Telephone No. (703)308-1235

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代理人(译)	清水初衷		
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摘要(译)

通过施用角霉素的水溶性衍生物来缓解疼痛的方法。 还提供了使用止痛剂的方法。