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Avaliação antinociceptiva de 2,3-substituídos-1,3-tiazolidin-4-onas mediante estimulo térmico em camundongos

Arthur Hipolito da Silva Neves

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“Triunfam aqueles que sabem quando lutar e quando esperar.”

Sun Tzu

Resumo

NEVES, Arthur Hipolito da Silva. **Avaliação antinociceptiva de 2,3-substituídos-1,3-tiazolidin-4-onas mediante estímulo térmico em camundongos.** 2015. 95f. Dissertação (Mestrado) – Programa de Pós-Graduação em Bioquímica e Bioprospecção, Centro de Ciências Químicas, Farmacêuticas e de Alimentos, Universidade Federal de Pelotas, Pelotas, 2015.

Para contribuir com o desenvolvimento de novas drogas analgésicas somado ao fato de que as 4-tiazolidinonas são um importante núcleo associado á muitas atividades farmacológicas, o presente estudo avaliou o potencial antinociceptivo de 2,3-substituídos-1,3-tiazolidin-4-onas mediante modelo de nocicepção aguda, induzida por estimulo térmico em camundongos. Os compostos sintetizados foram caracterizados por GC / MS e RMN de ^{1}H e ^{13}C e administrados na dose de 100 mg / kg (*ip*) (sal cloridrato). Dipirona de sódica (250 e 500 mg / kg; *ip*), cloridrato de tramadol (25 e 50mg / kg, *ip*) foram empregados como controles positivos. O teste da placa quente foi realizado em temperatura de 50 ± 0,1ºC e os animais avaliados nos tempos de 30, 60 e 90 minutos após a administração de drogas. Dos quatorze compostos testados, nove (**5Aa**, **5Ab**, **5Ac**, **5Ad**, **5Ba**, **5Bb**, **5Bd**, **5Ea**, **5Fa**) demostraram aumento significativo no tempo de latência quando comparados á solução salina (controle negativo), e três (**5Ab**, **5Ac** e **5Ad**) não demonstraram diferença significativa em comparação com dipirona sódica (500 mg / kg; *ip*) na avaliação de 30 minutos. Os maiores tempos de latência foram obtidos com a 3-(2-piperidin-1-il)etil)-tiazolidin-4-ona (**5Ab**, **5Ac** e **5Ad**). Além disso, os substituintes 2-butil (**b**), 2-fenil (**c**) e 2-ciclo-hexano (**d**) promoveram maior aumento no tempo de latência do que 4-fluorofenil (**a**). O composto 2-(4-fluorofenil)-3-(piridin-2-ilmetil)-tiazolidin-4-ona (**5Ea**) foi o único que manteve o efeito antinociceptivo na avaliação de 30, 60 e 90 minutos. Além disso, a substituição de 4-nitrofenil (**e**) ou 4-metoxifenil (**f**) não se demonstrou favorável para a atividade antinociceptiva destes compostos.

Palavras-chave: antinocicepção;4-tiazolidinonas; analgésico; dor aguda; teste da placa quente

Abstract

NEVES, Arthur Hipolito da Silva. **Antinociceptive evaluation of 2,3-substituted-1,3-thiazolidin-4-ones through thermal stimulation in mice.** 2015. 95f.Dissertation (Master Degree) – Programa de Pós-Graduação em Bioquímica e Bioprospecção, Centro de Ciências Químicas, Farmacêuticas e de Alimentos, Universidade Federal de Pelotas, Pelotas, 2015.

To contribute with the development of new analgesic drugs added to the fact that 4-thiazolidinones are an important scaffold associated with many pharmacological activity, the present study assessed the 2,3-substituted-1,3-thiazolidin-4-ones potential antinociceptive through the acute nociception model, induced by thermal stimulation in mice. The synthesized compounds were characterized by GC/MS and NMR of ^{1}H and ^{13}C and administered at dose 100 mg/kg (*ip*) (hydrochloride salt). Sodium dipyrone (250 e 500 mg/Kg; *ip*), tramadol hydrochloride (25 e 50mg/Kg; *ip*) was used with positive controls. The hot plate test was done at temperature of 50±0,1ºC and animals assess in times of 30, 60 and 90 minutes after administration drugs. From the fourteen compounds tested, nine (**5Aa**, **5Ab**, **5Ac**, **5Ad**, **5Ba**, **5Bb**, **5Bd**, **5Ea**, **5Fa**) showed significant increases in latency time when compared to saline (negative control), and three (**5Ab**, **5Ac** and **5Ad**) presented no significant difference compared to sodium dipyrone (500 mg/Kg; *ip*) in assess of 30 minutes. The highest latency times were obtained at the 3-(2-piperidin-1-yl)ethyl)thiazolidin-4-one derivatives (**5Ab**, **5Ac** and **5Ad**).Moreover, substituents 2-butyl (**b**), 2-phenyl (**c**) and 2-cyclohexane (**d**) promote greater increases in the latency time than 4-fluorophenyl (**a**). The compound2-(4-fluorophenyl)-3-(pyridin-2-ylmethyl)thiazolidin-4-one (**5Ea**) was the only that retained the antinociceptive effect in assessment of 30, 60 and 90 minutes. Moreover, the substitution of 4-nitrophenyl (**e**) or 4-methoxyphenyl (**f**) did not prove to be favorable for antinociceptive activity of these compounds.

Key-words: antinociception; 4-thiazolidinones; analgesic; acute pain; hot plate test

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AA - Ácido Araquidônico

AINEs - Anti-Inflamatórios Não Esteroidais

IASP - *International Association for the Study of Pain*

PG - Prostaglandinas

SNA - Sistema Nervoso Autônomo

SNC - Sistema Nervoso Central

WHO - *World Health Organization*

Sumário

1 INTRODUÇÃO

A percepção da dor representa um importante domínio da experiência humana, caracterizando um processo fundamental para a sobrevivência dos indivíduos, pois é o primeiro indicador de lesão tecidual (SILVA; RIBEIRO-FILHO, 2011). A dor, segundo a IASP (*International Association for the Study of Pain*), é definida como uma experiência sensorial e emocional desagradável associada a dano real ou potencial de tecidos ou descrita em termos de tais danos (KOPF; PATEL, 2010). Apresentando um componente sensorial (nociceptivo) e outro afetivo, estando o primeiro associado sua a percepção e o segundo a reação reflexa ou comportamental em busca de proteção contra o estímulo nocivo. Por fim, considera-se a dor como uma experiência genuinamente subjetiva e pessoal, a qual envolve elementos individuais como o gênero, condições socio-econômicas, o contexto etno-cultural, as percepções intelectuais e cognitivas, as quais podem modificar sua percepção (SILVA; RIBEIRO-FILHO, 2011; KOPF; PATEL, 2010).

Do ponto de vista temporal, a dor pode evoluir para um estado crônico, a qual persiste além do tempo normal de cicatrização tissular (MERSKEY; BOGDUK, 1994). Assim, tornando-se um quadro de dor contínua ou recorrente de duração mínima de três meses (DELLAROZA *et al*., 2008; HANNA, 2007).

Dados da WHO (*World Health Organization*) apontam que a dor crônica afeta 30% da população mundial, promovendo morbidade, afastamento da atividade laboral, incapacidade temporária ou permanente além de custos elevados aos sistemas de saúde (MONTINI; NEMAN, 2012). De fato, diversos estudos epidemiológicos ao redor do mundo revelam elevada prevalência de dor crônica em diferentes populações. Mediante inquérito postal, Bouhassira e cols (2008) estimaram a prevalência de dor crônica com ou sem características neuropáticas em 31,7% na população francesa. Estando a maior prevalência com características neuropáticas associada com a meia-idade (50-64 anos), profissões manuais e

moradores de áreas rurais. Já, no Japão, também mediante a inquérito postal estimou-se uma prevalência 40% de dor crônica na população, estando associada a problemas de saúde mental, redução da qualidade de vida e perda da vida social devido á ausência da atividade laboral (INOUE *et al*., 2015). Na Austrália, a dor crônica foi apontada por 17,1% dos homens e 20,0% das mulheres, tendo forte associação com idade avançada, sexo feminino, baixos níveis de escolaridade e ausência de plano de saúde complementar (BLYTH *et al*., 2001).

O Brasil também apresenta elevada de prevalência da dor crônica. Kreling e cols (2006) estimaram 61,4% de prevalência de dor crônica em trabalhadores da Universidade Estadual de Londrina (Paraná), sendo mais frequente no sexo feminino (69,2%) que no masculino (52,2%). Outro estudo estimou a prevalência de dor aguda nas regiões cervical, torácica ou lombar revelando que 34,1% dos adultos no sul do Brasil (Pelotas/RS) apresentaram dor alguma vez na semana anterior à entrevista (FERREIRA *et al*., 2011).

De fato os resultados acima demonstram o grande impacto da dor na sociedade contemporânea, incluindo a população em idade ativa. Portanto, a dor é considerada um importante problema de saúde publica, promovendo significativo impacto negativo na qualidade de vida, na atividade laboral diária (BLYTH *et al*., 2001; INOUE *et al*., 2015) e onerosos custos de tratamento (MONTINI; NEMAN, 2012; SLEED *et al*., 2005).

Nesse contexto, crítico para população deve-se racionalizar o uso de medicamentos para elevar o sucesso terapêutico, a fim de reduzir os impactos negativos da dor. Deste modo, deve-se considerar uma ampla e cuidadosa compreensão da natureza, dos diferentes tipos e padrões de dor (INCA, 2001), além da fundamentação racional para as intervenções terapêuticas e potenciais novos alvos de desenvolvimento de medicamentos (KOPF; PATEL, 2010).

Fármacos analgésicos opióides e não opióides estão entre os medicamentos mais prescritos e empregados no alivio da dor de etiologia multifatorial (CAMU; VANLERSBERGHE, 2002; CHANG *et al*., 2014). Entretanto, deve-se dimensionar os benefícios do tratamento com os potenciais riscos (CHOU *et al*.,2009), visto que os efeitos secundários desses agentes, usados cronicamente, comprometem a adesão do paciente ao tratamento farmacológico (CHANG *et al*., 2014; KURITA; PIMENTA, 2003). Assim, novas pesquisas buscam desenvolver fármacos mais eficazes e seguros, com reduzidos efeitos adversos, proporcionando a seus usuários maior

probabilidade de sucesso terapêutico. Novos fármacos com estas características podem ser desenvolvidos por meio da química medicinal e do planejamento molecular. Estas áreas com conhecimento, fundamentam-se no avanço do conhecimento dos processos fisiopatológicos, no melhor esclarecimento das vias bioquímicas e na seleção de alvos moleculares mais específicos e expressivos de forma a modelar fármacos inovadores por meio da otimização da estrutura molecular (GUIDO; ANDRICOPULO; OLIVA, 2010; VERÇOZA *et al*., 2009).

Entre os compostos atualmente estudados e empregados no âmbito da química medicinal, destacamos o heterociclo 4-tiazolidinona. Dados da literatura apontam o baixo custo de síntese, versatilidade dos processos de obtenção, ampla possibilidade estrutural e extenso espectro de atividades biológicas (LIESEM *et al*., 2008; TRIPATHI *et al*., 2014). Nesse contexto, há indícios relevantes da ação analgésica e anti-inflamatória de derivados 4-tiazolidinonas (TRIPATHI *et al*., 2014), inclusive promovendo a inibição de modo seletivo sobre a isoforma induzida da enzima ciclooxigenase (COX-2) (UNSAL-TAN *et al*., 2012; TARANALLI *et al*., 2008; VIGORITA *et al*., 2003). Essa inibição seletiva está associada a um melhor perfil farmacológico devido à menor incidência de efeitos adversos, pelo menos, de efeitos gastrointestinais (MENDES *et al*., 2012). Desse modo, tornando pertinente a investigação de 2,3-substituídas-1,3-tiazolidin-4-onas no que se refere à sua atividade antinociceptiva.

2 OBJETIVOS

Objetivo geral

Avaliar o potencial antinociceptivo de derivados 1,3-tiazolidin-4-ona em modelo de nocicepção aguda induzida por estimulo térmico em camundongos.

Objetivos específicos

- Avaliar o potencial antinociceptivo de seis derivados 2-(4-fluorfenil)-1,3-tiazolidin-4-ona no teste da placa quente.
- Selecionar dentre os compostos avaliados os que apresentaram melhor desempenho a fim de promover alterações estruturais na posição 2 do anel 1,3-tiazolidin-4-ona com o objetivo de aprimorar o desempenho.
- Avaliar o potencial antinociceptivo da segunda série de derivados 1,3-tiazolidin-4-ona no teste da placa quente.

3 REVISÃO DA LITERATURA

Diversos estudos relatam a elevada prevalência da dor em diferentes populações, caracterizando esta como um grave problema de saúde publica. Embora, um grande número de medicamentos estejam indicados e aprovados para o tratamento de diferentes algesias, fatores limitantes associados a efeitos adversos implicam diretamente na adesão ao tratamento e qualidade de vidas de seus usuários. Estas situações podem levar a uma adicional prescrição de outros medicamentos para manejo de efeitos adversos e/ou potencialização da terapia.

Assim, deve-se buscar desenvolver fármacos de modo que os efeitos adversos e interações medicamentosas não comprometam o tratamento farmacológico da dor ou de outras patologias associadas; logo sendo mais eficazes e seguros. Além disto, apresentem baixo custo de aquisição ao usuário ou ao gestor em saúde, com esquema posológico e forma farmacêutica conveniente não impondo dificuldades para administração.

O sucesso do tratamento da dor depende de uma cuidadosa avaliação da natureza, entendimento dos diferentes tipos e padrões de dor, assim como do conhecimento dos melhores tratamentos disponíveis (INCA, 2001).

Dor e nocicepção

Em 1979, a IASP (*International Association for the Study of Pain*) conceituou a dor como uma experiência sensorial e emocional desagradável associada a dano real ou potencial de tecidos ou descrita em termos de tais danos. Nesse contexto, torna-se implícito, junto à definição, que a dor nem sempre é resultado de um processo originário de lesão tecidual propriamente dita, mas também ocorrendo na ausência de lesões teciduais (KOPF; PATEL, 2010).

Ainda, a respeito da dor, considera-se como uma experiência genuinamente subjetiva e pessoal, sendo influenciada por elementos individuais do conhecimento humano (KOPF; PATEL, 2010). Vários fatores podem modificar a percepção da dor, tais como: as condições socioeconômicas, o contexto etnocultural o qual o sujeito

esta inserido, as suas percepções intelectuais ou cognitivas de experiências vividas (SILVA; RIBEIRO-FILHO; 2011; KOPF; PATEL, 2010). Logo, é imperativa a crença na queixa expressada de dor por parte do paciente, independente da opinião da equipe de saúde sobre a patologia, sendo este o princípio básico do tratamento correto e adequado da mesma (HANNA, 2007).

Deste modo, o processo de dor compreende dois elementos característicos: (*i*) o afetivo-emocional e (*ii*) a percepção discriminativa. O primeiro componente, afetivo-emocional, está relacionado com a ação ou o comportamento em busca de proteção contra o estímulo nocivo promotor da dor, e o segundo, percepção discriminativa, representa o componente nociceptivo relacionado à percepção da dor. Portanto, entende-se como nocicepção o componente fisiológico da dor, o qual envolve os processos de transdução, transmissão e modulação do estímulo nociceptivo (KLAUMANN *et al*., 2008).

Tendo como base os conceitos de dor e nocicepção, os termos como dor e analgesia são mais adotados aos seres humanos, enquanto nocicepção e antinocicepção são mais adotados aos animais, visto que os animais não são capazes de verbalizar os componentes subjetivos da dor (JONES, 1996; KLAUMANN *et al*., 2008).

De modo geral, a dor aguda ou crônica leva o indivíduo a manifestar sintomas como alterações nos padrões de sono, apetite e libido, manifestações de irritabilidade, alterações de energia, diminuição da capacidade de concentração. Especificamente no ponto de vista da dor crônica, os sintomas acima apresentam exacerbados levando á restrições no desenvolvimento das atividades familiares, profissionais e sociais (KRELING; CRUZ; PIMENTA, 2006).

Classificação da dor

A dor pode ser classificada segundo quatro pontos de vista: (*i*) temporal, (*ii*) fisiopatológico, (*iii*) da intensidade e (*iv*) etiológico, os quais apresentam relevância em relação ao tratamento (HANNA, 2007).

Sob o ponto de vista temporal a dor é principalmente classificada em aguda ou crônica, entretanto os termos episódica e subaguda também são descritos. A dor aguda apresenta localização e caráter bem definidos, podendo haver sinais de hiperatividade do sistema nervoso autônomo (SNA), enquanto o principal elemento da dor crônica é seu caráter persistente por três meses ou mais. A dor crônica

apresenta ainda como característica o desaparecimento dos sinais de hiperatividade do SNA, além de alterações significativas do estilo de vida, personalidade e capacidade funcional. Dessa forma, podemos nos referir a dor aguda como sintoma de determinada patologia, enquanto a dor crônica pode ser considerada o próprio processo patológico (HANNA, 2007).

Segundo a classificação fisiopatológica a dor pode ser somática, visceral ou neuropática. A dor somática é bem localizada e resulta da ativação de receptores periféricos e nervos sensitivos somáticos, enquanto a visceral apresenta ativação dos nociceptores viscerais e nervos viscerais, sendo difusa e frequentemente referida a áreas cutâneas. Já, a dor neuropática apresenta característica persistente geralmente descrita por queimação, alfinetadas ou formigamento, sendo resultado da estimulação direta do próprio tecido nervoso, seja este periférico ou central (HANNA, 2007).

Do ponto de vista da intensidade a dor pode ser graduada em (*i*) leve ou fraca, (*ii*) modera ou media, (*iii*) forte ou intensa e (*iv*) muito forte ou insuportável. (CARVALHO; KOWACS, 2006; HANNA, 2007). Contudo, como já foi descrito anteriormente, à informação da intensidade da dor é subjetiva, pois varia de acordo com experiências dolorosas anteriores, sexo, etnia, etc. Entretanto, pode ser mensuradas por escalas validadas a fim de avaliar a intensidade de dor com objetividade e especificidade quantitativa (Figuras 1) (CARVALHO; KOWACS, 2006).

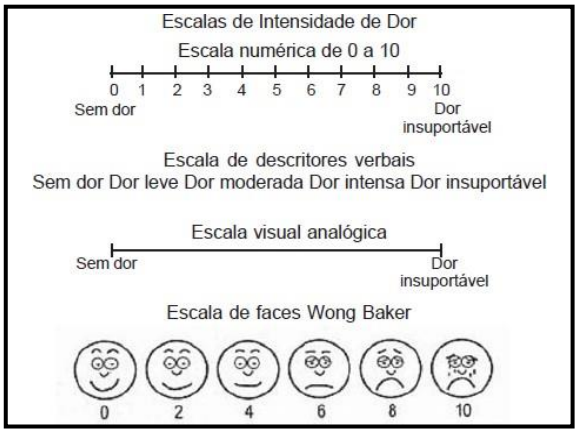

Figura 1: Escalas de avaliação da intensidade da dor.

Fonte: CARVALHO e KOWACS, 2006, p. 165.

A classificação etiológica também pode ser subdividida em dor (*i*) associada à lesão estrutural, como em quadros de dor oncológica, (*ii*) associada á transtornos psicofisiológicos que promovem dor, onde geralmente determinam alterações

funcionais crônicas as quais promovem dor mesmo após a resolução da doença de base, (*iii*) e por fim dor sem base estrutural e fisiológica aparente (HANNA, 2007).

Mecanismos neurais da dor

O termo nociceptor é a abreviatura de nocirreceptor, o qual corresponde a uma estrutura especializada na percepção de estímulos nocivos ou potencialmente nocivos (CATERIN; JULIUS, 1999). Estes promovem tradução do estímulo agressivo térmico, químico ou mecânico, em estímulo elétrico a qual será transmitido ao sistema nervoso central (SNC) e interpretado no córtex como dor (ROCHA *et al*., 2007).

Os nociceptores estão presentes nas terminações das fibras nervosas Aδ e C, e quando ativados, sofrem modificações na sua membrana, desencadeando o potencial de ação. Essa transformação do estímulo nocivo ou potencialmente nocivo em potencial de ação, no sentido das fibras periféricas para o SNC (ascendente), é o primeiro passo na sequência de eventos do fenômeno doloroso. As fibras Aδ, mielinizadas, transmitem o estímulo doloroso de forma rápida, enquanto as fibras C, não mielinizadas, promovem transmissão do estímulo de modo mais lento (ROCHA *et al*., 2007). Substâncias algiogênicas como acetilcolina, bradicinina, histamina, serotonina, leucotrienos, substância P, prostaglandinas (PG), interleucinas (IL) entre outras, presentes nos tecidos, promovem sensibilização dos nociceptores.

Na presença de um estímulo nocivo intenso, o qual promove lesão tissular ocorre uma sequência de eventos inflamatórios finalizado por um processo de reparação. Nesse contexto, ocorre liberação de enzimas para o meio extracelular, devido à ruptura de células. Essas enzimas atuam sobre os cininogênios formando cininas, que são pequenos polipeptídios da α_2-calicreína, presente no plasma e líquidos orgânicos. Logo, a calicreína, ativada pelo processo inflamatório, atua sobre a α-globulina, resultando na liberação de calidina, a qual é convertida de bradicinina por enzimas teciduais. A bradicinina promove vasodilatação arteriolar e aumento da permeabilidade vascular, colaborando para propagação do processo inflamatório (ROCHA *et al*., 2007).

Além disso, as enzimas liberadas em decorrência da lesão celular atuam sobre ácidos graxos de cadeia longa formando ácido araquidônico (AA), por meio da

fosfolipase A2,a qual é ativada mediante estímulos de origem química, inflamatória, traumática e miogênica (KUMMER; COELHO, 2002). A presença do AA inicia uma cascata metabólica mediada pela prostaglandina G/H síntetase, comumente chamada de COX, assim modulando a homeostase do processo inflamatório (MENDES *et al*., 2012). Existem três isoformas homólogas da COX (figura 2), COX-1 (constitutiva) COX-2(indutiva, com exceção do endotélio vascular), COX-3 (constitutiva no SNC). A constitutiva encontra-se presente na maioria dos tecidos atuando como citoprotetora gástrica, mantedora de homeostase renal e plaquetária, enquanto a COX-2 tem sua expressão aumentada em processos inflamatórios, na presença de citocinas, fatores do crescimento e estimulantes tumorais (KUMMER; COELHO, 2002).

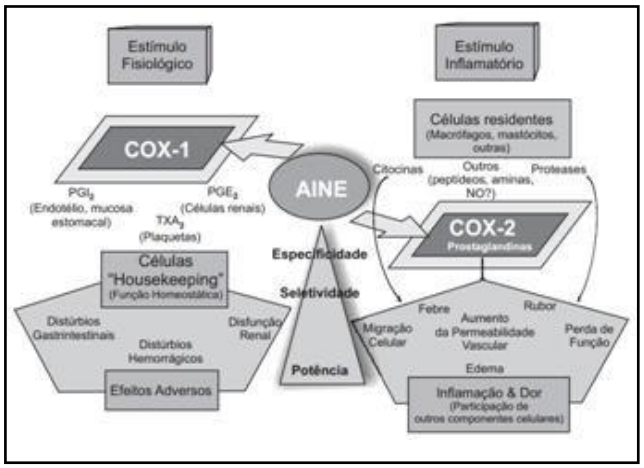

Figura 2: Ciclooxigenase na homeostase e inflamação.

Fonte: CARVALHO; CARVALHO; RIOS-SANTOS, 2004, p. 450.

A agressão tecidual, em geral, resulta no acumulo de metabólitos do AA, como prostaglandinas (PG) e leucotrienos, os quais incitam a degranulação de mastócitos, ativação direta de fibras nervosas, macrófagos e linfócitos. Ainda, tem-se a liberação de outros mediadores químicos, como o potássio, serotonina, substância P, histamina e cininas. Ocorrendo alterações na permeabilidade vascular, no fluxo sanguíneo local e produção dos sinais clássicos do processo inflamatório (rubor, calor, dor, tumor e perda funcional (ROCHA *et al*., 2007).

Além dessa resposta inflamatória tissular também ocorre uma resposta neurogênica, resultado em vasodilação, extravasamento de proteínas plasmáticas e ação sobre novas células inflamatórias com a liberação de mediadores químicos.

Por fim, inicia-se o processo de sensibilização periférica com exacerbação da resposta ao estímulo doloroso (ROCHA *et al*., 2007).

De modo geral, os mediadores do processo inflamatório atuam aumentando a sensibilidade dos nociceptores e reduzindo seu limiar de excitabilidade. Além do processo de sensibilização ocorre o fenômeno de modulação inibitória, mediado pelos receptores opióides periféricos, sendo seus agonistas endógenos a endofirna, encefalinas e as dinorfinas. Quando há persistência de reação inflamatória, a número de receptores opióides aumenta, indicando que a inflamação estimula o transporte axonal de receptores para periferia (ROCHA *et al*., 2007).

A transmissão dos estímulos nocivos através dos circuitos intramedulares não é um processo passivo, logo apresentam a capacidade de alterar o estímulo e a resposta dolorosa. Desse modo, a interação entre esses circuitos determinará se a mensagem relacionada ao estímulo nocivo ascenderá até o córtex cerebral (ROCHA *et al*., 2007).

No corno dorsal da medula espinhal ocorre o processo de sinapse entre os neurônios aferentes primários (SNP) com os neurônios secundários na medula espinhal (SNC). As fibras nociceptivas ascendem pelos tratos espinotalâmicos, espinorreticular, espinomesencefálico, coluna dorsal pós-sináptica e sistema espinoamigdaliano, assim transmitindo informações sobre dor e temperatura. Algumas dessas fibras ascendem até o tálamo e córtex cerebral, sendo estas as regiões finais das vias de nocicepção, enquanto o tálamo informa a existência de sensação nociceptiva o córtex discrimina o tipo de sensação (ROCHA *et al*., 2007).

Mediadores químicos da dor

Muitos mediadores químicos atuam de forma direta ou indireta no processo nociceptivo, sendo responsáveis pela complexidade de eventos que ocorrem na transmissão do estímulo nocivo, tanto no sistema nervoso periférico (SNP) como central (SNC). Esses mediadores podem ser metabólitos do ácido araquidônico (prostanóides), aminoácidos ou derivados (glutamato, noradrenalina, serotonina e dopamina), peptídeos (cininas, taquicininas), proteínas (citocinas) entre outros (ROCHA *et al*., 2007).

Tratamento farmacológico da dor

O tratamento da dor, principalmente aquela de difícil controle, compreende medidas de caráter farmacológico, métodos não farmacológicos e procedimentos invasivos em situações específicas. Entre os métodos não farmacológicos destacam-se o uso da terapia cognitivo-comportamental, fisioterapia e acupuntura, enquanto o método farmacológico apresenta o amplo e bem difundido uso de três grupos de medicamentos: (*i*) analgésicos opióides, (*ii*) analgésicos não opióides e (*iii*) analgésicos adjuvantes (co-analgésicos). Sendo, seu emprego orientado principalmente pelo tipo e intensidade de dor (FRANCO *et al*., 2011, HANNA, 2007).

Em, 1976 a WHO propôs a organização do tratamento farmacológico da dor por meio da utilização de uma escada analgésica (Figura 3), a qual orienta o uso sequêncial dos medicamentos de acordo com intensidade da dor apontada pelo paciente. Esta escada é composta por três degraus, onde os medicamentos devem ser administrados preferencialmente por via oral. Contudo, foi proposto a inclusão de um quarto degrau onde são alocados os procedimentos invasivos, como os bloqueios anestésicos e os procedimentos cirúrgicos (INCA, 2001; RABELO; BORELLA, 2013).

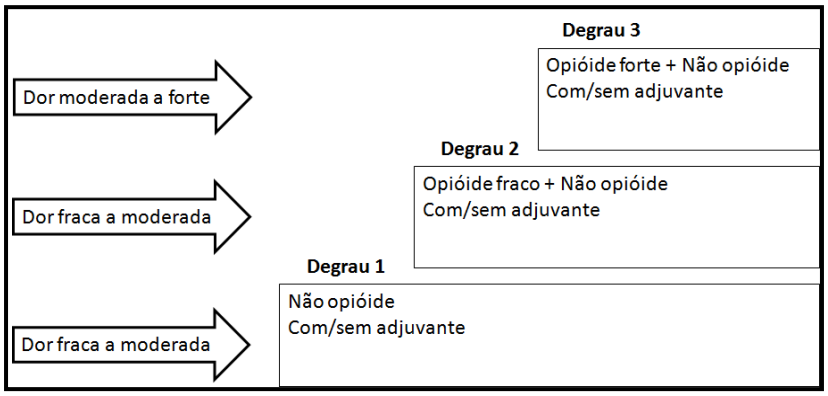

Figura3: Escada analgésica da *World Health Organization* (WHO)

A associação desses grupos de fármacos promovem um mecanismo sinérgico a fim de potencializar a terapia medicamentosa, denominado de analgesia multimodal. Esta visa à associação de diferentes classes de medicamentos com distintos mecanismos de ação, proporcionando um melhor efeito analgésico e reduzindo os efeitos adversos, pois permite diminuir a dose total de cada medicamento empregado no tratamento (CLIVATTI; SAKATA; ISSY, 2009). Assim, os analgésicos não opióides previnem a sensibilização de receptores periféricos e

centrais da dor através da inibição da COX (KUMMER; COELHO, 2002), enquanto os fármacos opióides promovem inibição da transmissão periférica e central da via nociceptiva aferente, devido ao agonismo dos receptores opióides (KLAUMANN *et al*., 2008). Por fim, os analgésicos adjuvantes promovem a otimização da analgesia promovida pelos outros fármacos (RIBEIRO; SCHMIDT; SCHMIDT; 2002).

Analgésicos opióides

Analgésicos opióides (Tabela 1) representam grupo de fármacos naturais, semi-sintéticos ou sintéticos amplamente utilizados no manejo da dor (KLAUMANN *et al*., 2008). São indicados no tratamento de dores agudas, moderadas ou intensas não responsivas ao outros analgésicos, sendo eficientes no tratamento da dor inflamatória aguda (KLAUMANN *et al*., 2008; KRAYCHETE *et al*., 2013). Ainda, representam a base da terapia farmacológica em quadros de dor crônica de intensidade modera a intensa (KRAYCHETE *et al*., 2013; MARTIN; EISENACH, 2001). No entanto, não são igualmente eficazes para todos os tipos de dor, pois aquelas de etiologia neuropática os analgésicos opióides apresentam resposta pobre ou de curta duração comprometendo o tratamento (KLAUMANN *et al*., 2008).

Tabela 1: Analgésicos opióides

	Fármaco	Posologia	Considerações
Opióide fraco	Codeína	VO/EV: 30 – 60 mg (4/4h)	Muito utilizado em associação com paracetamol 500mg. Frequentemente é o primeiro fármaco utilizado.
	Tramadol	VO/EV: 50 – 100 mg (4/4 – 6/6h)	Mecanismo de ação misto opióide e inibidor da recaptação de catecolaminas. Sinergismo com os AINEs.
Opióide forte	Morfina	VO: 60 mg (4/4h) EV: 10 mg (4/4h)	Rápido inicio de ação.
	Fentanil	VT: 25 – 300µg (72-72h)	Início de ação demorado. Não é recomendado como escolha inicial.

VO: Via Oral; EV: Via Endovenosa; VT: Via Transdérmica.

Fonte: Adaptado de HANNA, 2007.

Esses fármacos atuam a nível celular ligando-se aos receptores opióides (MOP, KOP, DOP e NOP) presentes no SNC, especialmente no núcleo do trato solitário, área cinzenta periaquedutal, córtex cerebral, tálamo e substância gelatinosa da medula espinhal. Receptores opióides podem também estar presentes em terminações nervosas aferentes periféricas e em diversos outros órgãos

(TRIVEDI; SHAIKH; GWINNUTT, 2007). Na presença de inflamação podem se expressar no sistema nervoso periférico, assim possibilitando o uso tópico destes medicamentos (RIBEIRO; SCHMIDT; SCHMIDT, 2002).

Embora, os analgésicos opióides sejam muito efetivos sua utilização é comprometida pelo fato de apresentarem muitos efeitos indesejados em diversos sistemas orgânicos, como SNC, cardiovascular, respiratório, gastrointestinal entre outros (TRIVEDI; SHAIKH; GWINNUTT, 2007). Desse modo, a rotação dos analgésicos opióides pode reduzir os efeitos adversos e/ou aliviar a dor de forma adequada em 50 a 70% dos pacientes (KRAYCHETE *et al*., 2013). A rotação corresponde à troca de um opióide por outro sendo adequada devido ao declínio na eficácia, ou quando a analgesia está associada a efeitos adversos que comprometem a qualidade de vida (KRAYCHETE *et al*., 2013).

No SNC sedação, dificuldade de concentração e sonolência são efeitos adversos comuns, embora o alívio da dor possa ocasionar sono. Ainda, promovem uma sensação de bem estar (euforia), contudo na ausência de dor, podem ocasionar agitação e inquietação (disforia). Ainda podem ocorrer alucinações, principalmente após o uso de opióides agonistas KOP. Por fim, quadros de tolerância e dependência também são descritos e de importante implicação clínica (TRIVEDI; SHAIKH; GWINNUTT, 2007).

Em relação ao sistema cardiovascular promovem bradicardia discreta devido à redução do tônus simpático e efeito direto sobre o nó sinoatrial. Podem acarretar quadros hipotensivos, principalmente quando associados à hipovolemia, ocorrendo em virtude da vasodilatação periférica causada pela liberação de histamina e redução do tônus simpático (TRIVEDI; SHAIKH; GWINNUTT, 2007).

No sistema respiratório, a depressão respiratória é o sinal mais relevante, sendo mediada pelos receptores MOP localizados no centro respiratório do tronco cerebral. Desse modo, tem-se redução da frequência respiratória e a dessensibilização dos quimiorreceptores centrais às alterações de pressão parcial de dióxido de carbono (PCO2). Assim, o uso de opióides associados a fármacos depressores do SNC, como benzodiazepínicos podem agravar a depressão respiratória. Por fim, altas doses de opióides podem ocasionar rigidez muscular generalizada, especialmente na musculatura da parede torácica, o que pode interferir na ventilação (TRIVEDI; SHAIKH; GWINNUTT, 2007).

Sobre o sistema gastrointestinal, ocorre à ativação de receptores opióides localizados na zona quimiorreceptora de gatilho do vômito, dessa forma podem desencadear náuseas e vômitos. A constipação ocorre devido ao aumento do tônus da musculatura lisa e redução da motilidade, o que resulta no retardo da absorção e aumento da pressão no sistema biliar (TRIVEDI; SHAIKH; GWINNUTT, 2007).

Analgésicos não opióides

Os analgésicos não opióides (Tabela 2) são representados principalmente pelos agentes anti-inflamatórios não esteroidais (AINEs), estando entre as drogas mais prescritas e utilizadas no mundo (BATLOUNI, 2010; CAMU; VANLERSBERGHE, 2002). Esses, em decorrência do seu mecanismo de ação exercem atividade antiinflamatória, analgésica e antipirética, sendo considerados os agentes de escolha no tratamento da dor de intensidade leve a moderada(HANNA, 2007). Além disso, podem ser empregados em associação a outros fármacos, de modo a reduzir as doses analgésicas e a incidência de efeitos adversos (KUMMER; COELHO, 2002).

Tabela 2: Analgésicos não opióides

Fármaco	Posologia	Dose máxima mg/24 h	Considerações
Paracetamol	VO: 500 – 1000 mg (4/4 – 6/6 h)	4000	Hepatotóxico. Frequentemente associado à opióide. Útil em pacientes com distúrbio da coagulação. Efeito anti-inflamatório ausente.
Dipirona	VO: 500 mg IM/EV: 1000 mg (4/4 – 6/6 h)	4000 (VO) 3000 (EV)	Erupção cutânea, hipotensão arterial. Agranulocitose (casos raros). Efeito anti-inflamatório ausente
Ibuprofeno	VO: 60 mg (4/4 h) EV: 10 mg (4/4 h)	3200	Erupções cutâneas. Irritação gástrica.
Diclofenaco	VO/IM: 50 – 75 mg (8/8 h)	300	Inicio de ação lento. Pode levar a redução do fluxo sanguíneo renal. Irritação gástrica.
Celecoxibe	VO: 100 – 200 mg (12/12 h)	400	Menor toxicidade gastrointestinal, hematológica e renal.

VO: Via Oral; EV: Via Endovenosa; IM: Via Intramuscular.

Fonte: Adaptado de HANNA, 2007.

Os AINEs exercem efeito supressor no processo inflamatório através da inibição da COX, assim impedindo a conversão do ácido araquidônico (AA) em prostaglandinas (PG) (CARVALHO; CARVALHO; RIOS-SANTOS, 2004), as quais

promovem, entre outras funções, sensibilização central e periférica a dor (KUMMER; COELHO, 2002). Em relação à inibição das isoformas da COX, podem ocorrer de modo não seletivo através do uso de AINEs convencionais ou de modo seletivo pelo uso dos designados inibidores seletivos da COX-2 (Tabela 3) (BATLOUNI, 2010; MONTEIRO *et al*.,2008).

Tabela 3: Classificação atual dos AINEs

Inibidores seletivos da COX-1	***Inibidores seletivos da COX-2***
Ácido acetilsalicílico (em baixas doses).	Meloxicam, etodolaco, nimesulida.
Inibidores não seletivos da COX	***Inibidores altamente seletivos da COX-2***
Ácido acetilsalicílico (em altas doses), piroxicam, indometacina, diclofenaco, ibuprofeno.	Celocoxibe, etoricoxibe.

Fonte: Adaptado de MONTEIRO *et al*.,2008.

Em relação aos efeitos adversos, o uso de AINEs convencionais por períodos prolongados torna-se limitado devido ao desenvolvimento de efeitos gastrintestinais (Tabela 4) como dispepsia, dor abdominal, sangramento, úlcera e perfusão gástrica ou duodenal (CARVALHO; CARVALHO; RIOS-SANTOS, 2004) oriundos do bloqueio inespecífico da COX (KUMMER; COELHO, 2002). Em relação à função renal os AINEs são frequentemente associados a edema e retenção de sal, além outras complicações como hipercalcêmica, hiponatremia ou mesmo falência renal aguda são descritas.

Tabela 4: Efeitos adversos gastrointestinais dos AINEs

Efeitos leves	Dispepsia. Erosões gastrointestinais.
Efeitos moderados	Anemia ferropriva. Úlceras gastrointestinais.
Efeitos graves	Sangramento gastrointestinal severo. Perfuração aguda. Obstrução gástrica.

Fonte: Adaptado de MONTEIRO *et al*., 2008.

Os inibidores seletivos da COX-2 são citados como alternativas seguras para usuários com maior risco de desenvolver úlcera péptica ou hemorragia gastrointestinal. Contudo, os efeitos adversos cardiovasculares e renais desses agentes seletivos podem limitar seu uso (CARVALHO; CARVALHO; RIOS-SANTOS, 2004; JOSE, 2014).

Analgésicos adjuvantes

Os analgésicos adjuvantes (Tabela 5) representam um grupo heterogêneo de medicamentos, empregados no processo de analgesia multimodal (RIBEIRO; SCHMIDT; SCHMIDT; 2002). Quando associados à terapia farmacológica otimizam o tratamento analgésico, pois permitem reduzir a dose empregada dos demais medicamentos (CLIVATTI; SAKATA; ISSY, 2009), assim atuando de forma a aumentar a analgesia (anticonvulsivantes e antidepressivos), controlar e/ou reduzir os efeitos adversos (laxativos e antieméticos) e modular os sintomas que contribuem para a dor do paciente (antidepressivos, ansiolíticos, indutores do sonos)(HANNA, 2007; SANTOS; SOUZA, 2010).

A cafeína é uma metilxantina com efeitos estimulantes que apresenta efeito analgésico aditivo devido ao bloqueio dos receptores de adenosina, sendo amplamente encontrada em associação com AINEs, principalmente o paracetamol (GODOY; GONÇALVES; MORAES, 2012). A cafeína é útil no manejo de dor aguda por aumentar o efeito analgésico do paracetamol e devido à analgesia direta promovida pela cafeína. Em situações de dor crônica seu uso é limitado pela incidência dos efeitos adversos, abstinência e risco de dependência, embora melhore a performance cognitiva de pacientes em uso de morfina (GODOY; GONÇALVES; MORAES, 2012; TAVARES; SAKATA, 2012).

O uso de fármacos antiepiléticos, como a gabapentina, é bem difundido e atuam mediante a redução hiperexcitabilidade induzida por lesão de neurônios do corno posterior, que é responsável pela sensibilização central (MANEUF *et al*., 2003). Ainda, o uso de gabapentina tem sido reportado no tratamento de dores agudas ou crônicas, sendo um fármaco seguro, eficaz e bem tolerado (CHANG *et al*., 2014; CLIVATTI; SAKATA; ISSY, 2009).

Tabela 5: Analgésicos adjuvantes

Classe	Fármaco	Dose (24h)	Considerações
Antidepressivos	Amitriptilina	10 – 150 mg	Iniciar com doses baixa. Aumentar dose gradualmente.
	Paroxetina	25 – 150 mg	
Anticonvulsivantes	Carbamazepina	200 – 1200 mg	Útil na neuralgia do trigêmeo.
	Gabapentina	300 – 4800 mg	Iniciar com 300mg/24h Primeira escolha na dor neuropática

Antipsicóticos	Haloperidol	1 – 15 mg	Efeitos adversos extrapiramidais.
	Olanzapina	2,5 – 20 mg	Sedação e ganho de peso.
Corticóides	Prednisona	5 – 60 mg	Efeitos anti-inflamatórios, analgésicos e antieméticos.
	Dexametasona	0,5 – 16 mg	
Anti-histamínicos	Hidroxizina	25 – 100 mg	Sinergismo com opióides. Efeito antiemético e sedativo.

Fonte: adaptado de HANNA, 2007

Derivados 4-tiazolidinonas

A pesquisa de novos compostos com atividade analgésica é justificada pela alta prevalência de quadros dolorosos, seja agudo ou crônico, e pela necessidade de desenvolver fármacos mais seguros e eficazes. Visto a grande possibilidade de efeitos adversos que podem comprometer o tratamento farmacológico.

O núcleo 4-tiazolidinona ou 1,3-tiazolidin-4-ona é um heterocíclico de cinco membros, apresentando um átomo de enxofre na posição 1, um átomo de nitrogênio na posição 3 e um grupo carbonila na posição 4 (Figura 3) (CUNICO *et al*., 2008). Sendo, as posições 2, 3 e 5 do anel passíveis de substituição, em geral, por outros grupos químicos de forma a alterar os parâmetros químicos e biológico desses compostos, já que isoladamente este núcleo não apresenta atividade biológica (CUNICO *et al*., 2008; LIESEN *et al*., 2008).

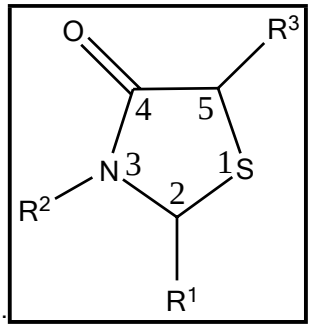

Figura 4: Estrutura geral das 1,3-tiazolidin-4-onas

No campo da química medicinal o núcleo 4-tiazolidinona apresenta grande importância devido as suas muitas atividades biológicas reportadas na literatura (LIESEN *et al*., 2008). Atualmente, apresentam o núcleo 4-tiazolidinona os medicamentos pioglitazona, ralitoline e o etozoline, os quais atuam como hipoglicemiante, antiepilético e anti-hipertensivo, respectivamente (JAIN *et al*., 2012). Além disso, são encontrados diversos estudos que relatam as atividades

farmacológicas das 4-tiazolidinonas, tais como antimicrobiana (DEEP *et al*., 2014), antiviral (JADAV *et al*., 2015), anti-hiperglicêmica (BHOSLE, *et al*., 2014), anti-epilética (DWIVEDI *et al*., 2012), anti-histamínica (VITTORIA *et al*., 1992) antioxidante (RANGANATHA *et al*., 2014), inibidores da degradação da cartilagem (PANICO *et al*., 2011), analgésica e anti-inflamatória (SINGH *et al*., 2015) entre outros.

Estudos recentes demonstram que a atividade antinociceptiva dos derivados 4-tiazolidinonas provêm da inibição da COX, incluindo a inibição seletiva da COX-2, desse modo exercendo tanto atividade analgésica como anti-inflamatória. Contudo, a presença que formas isoméricas podem influenciar a atividade desses compostos. (GERONIKAKI *et al*., 2008; TARANALLI *et al*., 2008; UNSAL-TAN *et al*., 2012; VIGORITA *et al*., 2003).

Vigorita e cols (2001) investigaram a atividade analgésica de 3,3'-(1,2-etanodiil)-bis[2-aryl-4-tiazolidinonas] nas doses 100 mg/kg administradas por via oral utilizando modelos animais o teste de contorções abdominais induzidas pelo ácido acético e o teste da placa quente. Os derivados 2,4-dimetoxifenil (Figura 6a) apresentaram o melhor perfil farmacológico em ambos os testes seguidos pelos análogos estruturais 3,4-diclorofenil (Figura 6b). Além disso, todos os compostos avaliados nesse estudo apresentaram efeitos ulcerogênicos e toxicidade aguda inferior à indometacina e fenilbutazona. Por fim, demonstrou considerável diferença na atividade dos compostos em relação à assimetria R ou S, dependendo do arranjo espacial.

Posteriormente, Vigorita e cols (2003) investigaram o efeito da quiralidadede3,3'-(1,2-etanediil)-bis[2-(3,4-dimetoxyfenil)-4-tiazolidinonas] sobre a seletividade das ciclo-oxigenases (COX-1 e COX-2) *in vitro* demonstrando que os anantiómeros SS possuem maior perfil seletividade sobre a COX-2.

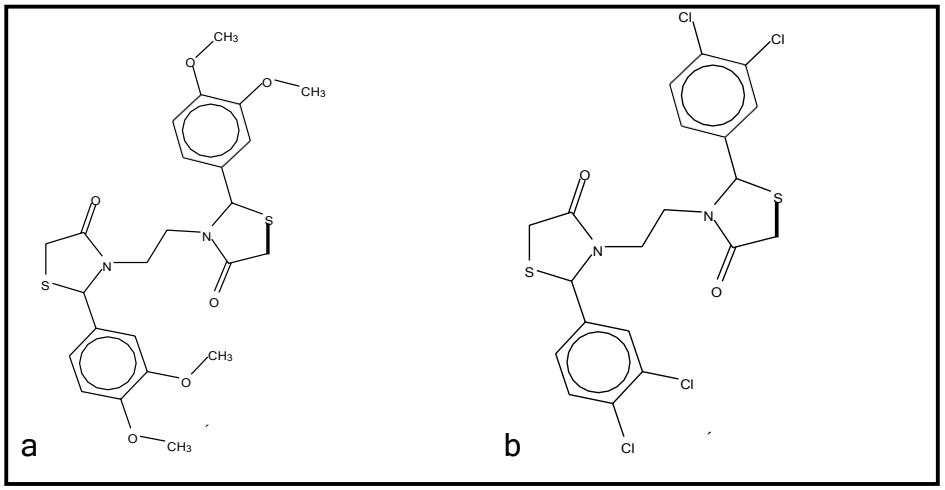

Figura 5:3,3'-(1,2-etanodiil)-bis[2-aryl-4-tiazolidinonas]

Fonte: VIGORITA *et al*., 2001, p. 2721.

Em estudo recente derivados 4-tiazolidinonas sintetizados a partir de sulfanilamida (Figura 6)foram avaliados quanto sua atividade farmacológica. Esses derivados exibiram excelentes efeitos anti-inflamatório, analgésico e antipiréticonas doses de 100 mg/kg (VO) em todos os modelos. Compostos contendo, 4-metoxifenil, 3-cloro-4-hidrofenil, 4-clorofenil e 4-fluorfenil na posição 2 do anel 4-tiazolidinona demonstraram inibição máxima da atividade de COX-2 sem inibir a COX-1. Ainda, segundo os autores, a presença de grupos funcionais Cl, OCH_3, NO_2 e OH no anel aromático resulta em aumento da atividade em comparação com aos compostos não substituídos (TARANALLI *et al*., 2008).

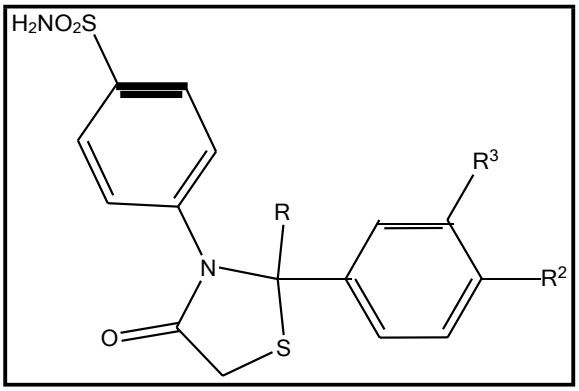

Figura 6: Estrutura geral dos compostos estudados por Taranalli e cols.

Fonte: TARANALLI *et al*., 2008.

Portanto, torna-se pertinente avaliar o potencial antinociceptivo de 2,3-substituídos-1,3-tiazolidin-4-onas. Com base nos estudos anteriores, nossas estruturas iniciais apresentaram grupo 4-fluorfenil na posição 2 do anel 4-

tiazolidinona, enquanto a posição 3 mantivemos o nitrogênio como um heteroátomo do substituinte. .

4 MANUSCRITO

Manuscrito a ser submetido na European Journal of Medicinal Chemistry

The antinociceptive evaluation of 2,3-substituted-1,3-thiazolidin-4-ones through thermal stimulation in mice

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Abstract

To contribute with the development of new analgesic drugs added to the fact that 4-thiazolidinones are an important scaffold associated with many pharmacological activity, the present study assessed the 2,3-substituted-1,3-thiazolidin-4-ones potential antinociceptive through the acute nociception model, induced by thermal stimulation in mice. The synthesized compounds were characterized by GC/MS and NMR of ^{1}H and ^{13}C and administered at dose 100 mg/kg (*ip*) (hydrochloride salt). Sodium dipyrone (250 e 500 mg/Kg; *ip*), tramadol hydrochloride (25 e 50mg/Kg; *ip*) was used with positive controls. The hot plate test was done at temperature of 50±0,1ºC and animals assess in times of 30, 60 and 90 minutes after administration drugs. From the fourteen compounds tested, nine (**5Aa**, **5Ab**, **5Ac**, **5Ad**, **5Ba**, **5Bb**, **5Bd**, **5Ea**, **5Fa**) showed significant increases in latency time when compared to saline (negative control), and three (**5Ab**, **5Ac** and **5Ad**) presented no significant difference compared to sodium dipyrone (500 mg/Kg; *ip*) in assess of 30 minutes. The highest latency times were obtained at the 3-(2-piperidin-1-yl)ethyl)thiazolidin-4-one derivatives (**5Ab**, **5Ac** and **5Ad**). Moreover, substituents 2-butyl (**b**), 2-phenyl (**c**) and 2-cyclohexane (**d**) promote greater increases in the latency time than 4-fluorophenyl (**a**). The compound 2-(4-fluorophenyl)-3-(pyridin-2-ylmethyl)thiazolidin-4-one (**5Ea**) was the only that retained the antinociceptive effect in assessment of 30, 60 and 90 minutes. Moreover, the substitution of 4-nitrophenyl (**e**) or 4-methoxyphenyl (**f**) did not prove to be favorable for antinociceptive activity of these compounds.

Keywords: antinociception, 4-thiazolidinone derivatives, analgesic, acute pain, hot plate test

1 Introduction

In 1979, the *International Association for the Study of Pain* (IASP) defined pain as “an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage,” which is still valid today [1]. For pain treatment, non-pharmacological, pharmacological and invasive methods are used [2], so three classes of analgesic drugs may be described, opioid analgesic, non-steroidal anti-inflammatory (NSAIDs) and adjuvant analgesic, such as antidepressants, anticonvulsants and anxiolytics medications. The combination of these analgesic drugs are very useful for chronic pain therapy [2-4].

The use of systemic analgesic is the most commonly used method for relief of acute pain cases [5]. However, independently for the pain type, acute or chronic, the pharmacological therapy based on opioids and NSAIDS promote several side effects, such as abuse, overdose, tolerance, dependence, sedation, gastrointestinal dysfunction, among others. The patients sometimes require other medication for controlling and managing these side effects [6, 7], thereby decreasing the adherence to pharmacological treatment [8]. Thus, researchers shall develop new drugs for pain treatment rather safer and more effective, with side effect reduction that limits medication use, promoting to the user larger therapeutic success [9].

In this sense, the 1,3-thiazolidin-4-ones ring has acquire great importance inside science community due to its multiple biological actions [10, 11]. Accordingly, researchers have established actions opposed germs and other infectious agents (virus, bacteria, fungi and parasites), against chronic diseases as diabetes, hyperlipidemic and convulsion, as well as the presence of antiinflammatory and analgesic effect, among others [11, 12]. Moreover, these 1,3-thiazolidin-4-ones derivative have low cost and large synthesis versatility, and also large structural variety depending on the adopted synthesis method [10, 12].

The 1,3-thiazolidin-4-one ring is a five-membered heterocyclic, that has a sulfur atom at position 1, a nitrogen at position 3 and a carbonyl group at position 4[11]. There are usually substituents in the 2, 3 and 5-position of the ring, which promote changes in chemical, physical and biological parameters [10, 12].

Therefore, taking advantage of the experience of our group in the synthesis of these derivatives and seeking to contribute to the development of new drugs for pain management and reduction of the negative outcome of the disease, the present

study evaluated the 2,3-substituted-1,3-thiazolidin-4-ones potential antinociceptive through the acute nociception model, induced by thermal stimulation (hot plate test) in mice.

2 Material and Methods

Chemistry

All common reagents and solvents were used and obtained from commercial suppliers without further purification. Reactions progress was monitored by a thin-layer chromatography (TLC) (hexane:ethyl acetate 3:1) and/or by a Shimadzu Gas Chromatograph GC-2010, HP-1 column (cross linked methyl siloxane, 30 m × 0.32 mm × 0.25 μm): Column head pressure, 14 psi, program: T0 = 60 °C; t0 = 2.0 min; rate 10.0 °C min-1; Tf = 280 °C; tf = 13.0 min; Inj. = 250 °C; Det. = 280 °C. The melting points were determined using open capillaries on a Fisatom model 430 apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker DRX 400 spectrometer (^{1}H at 400 MHz and ^{13}C at 100 MHz), on a Bruker Avance 500 spectrometer (^{1}H at 500 MHz and ^{13}C at 125 MHz) or on a Bruker AC-200F spectrometer (^{1}H at 200 MHz and ^{13}C at 50 MHz) in CDCl3 or D20 containing TMS as an internal standard. The mass spectra were obtained on a Shimadzu GCMS-QP2010SE with a split-splitless injector and equipped with a RDX-SMS capillary column (30 m x 0.25 mm x 0.25 μm); helium was used as the carrier gas (56 kPa).

General procedure for the synthesis of thiazolidinones **4Aa-f**, **4Ba-d**, **4Ca-Fa**.

The compounds**4Aa**, **4Ac** (WO 03\008398 A1, 2003), **4Ae** and **4Af** were obtained according Kunzler *et al*, 2013[14].The compounds **4Ea** and **4Fa** were obtained according Gouvea *et al*, 2012 [13].

For the synthesis of novel compounds, at first, the reaction occurs through a solution of 1 mmol of amine 1 and 1 mmol of aldehyde or ketone 2 in refluxing toluene (30 ml) for two hours using a Dean-Stark trap. After than 3 mmol of mercaptoacetic acid 3 was added and the mixture was heated for more 3 hours. The organic layer was washed with saturated $NaHCO_3$ (3 x 30 ml), dried with $MgSO_4$ and concentrated on rotary evaporator to give the products. All products were purified by

column chromatography using hexane:ethyl acetate (7:3) as eluent. The one pot methodology and the reaction conditions were in agreement with recent paper published by our research group [14]. The atom-numbering of compounds **4A-D** and **5A-D** for NMR analyses identification are given in Figure 1.

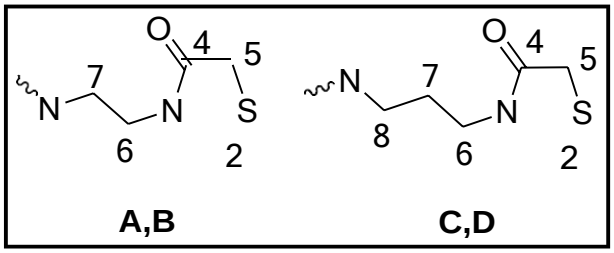


Figure 1: The atom-numbering for compounds 4A-D and 5A-D.

2-butyl-3-(2-(piperidin-1-yl)ethyl)thiazolidin-4-one **4Ab**.

Oil; ^{1}H NMR δ ($CDCl_3$, 400MHz, ppm, J_{H-H} = Hz); 4.76 (dt, 1H, ^{3}J=8.5, ^{4}J=2.3, H-2); 3.74 (ddd, 1H, ^{2}J=13.7, ^{3}J=7.7, ^{3}J=6.0, H-6a); 3.50 (dd, 1H, ^{2}J=15.5, ^{4}J=1.5, H-5a); 3.41 (d, 1H, ^{2}J=15.5, H-5b); 3.04 (dt, 1H, ^{2}J=13.9, ^{3}J=6.9, H-6b); 2.47 (ddd, 1H, ^{2}J=13.4, ^{3}J=7.3, ^{3}J=6.5, H-7a); 2.35 (ddd, 1H, ^{2}J=12.8, ^{3}J=7.1, ^{3}J=5.8, H-7b); 2.33-2.39 (m, 4H); 1.81-1.89 (m, 1H); 1.56-1.63 (m, 1H); 1.46-1.52 (m, 4H); 1.33-1.38 (m, 2H); 1.24-1.32 (m, 4H); 0.82-0.87 (m, 3H). ^{13}C NMR δ ($CDCl_3$, 100MHz, ppm, J_{C-F} = Hz); 171.0 (C-4); 62.0 (C-2); 56.1 (C-7); 54.6 (2C); 39.8 (C-6); 35.2; 32.0 (C-5); 26.3; 25.8 (2C); 24.1; 22.3; 14.0. MS (70 eV): m/z (%) = 270 (M^+, 1); 111 (3); 102 (1); 98 (100); 84 (2).

2-(4-fluorophenyl)-3-(2-(pyrrolidin-1-yl)ethyl)thiazolidin-4-one **4Ba**.

M.p.: 56-58 ºC; ^{1}H NMR δ ($CDCl_3$, 200MHz, ppm, J_{H-H} = Hz); 7,26-7,34 (m, 2H, Ar); 7,02-7,12 (m, 2H, Ar); 5.86 (s, 1H, H-2); 3,75- 3,88 (m, 2H, H-5a, H-6a); 3.71 (d, 1H, ^{2}J=15.5, H-5b); 2,65-2,88 (m, 2H, H-6b, H-7a); 2,46-2,60 (m, 5H, H-7b, H-8); 1,74-1,80 (m, 4H). ^{13}C NMR δ ($CDCl_3$, 50MHz, ppm, J_{C-F} = Hz); 171.3 (C-4); 162.9 (d, ^{1}J= 247.7, Ar),135.3 (d, ^{4}J= 3.2, Ar), 129.1 (d, 2C, ^{3}J= 8.5, Ar), 116.0 (d, 2C, ^{2}J= 21.8, Ar); 63.2 (C-2); 54.0 (2C, C-8); 52.9 (C-7); 41.2 (C-6); 32.9 (C-5); 23.4 (2C). MS (70 eV): m/z (%) = 294 (M^+, 2); 292 (M-4, 0.5); 224 (0.5); 153 (1); 139 (2); 97 (6); 84 (100); 69 (3).

2-butyl-3-(2-(pyrrolidin-1-yl)ethyl)thiazolidin-4-one **4Bb**.

Oil; ^{1}H NMR δ ($CDCl_3$, 400MHz, ppm, J_{H-H} = Hz); 4.70 (dt, 1H, ^{3}J=8.5, ^{4}J=2.3, H-2); 3.77 (ddd, 1H, ^{2}J=14.2, ^{3}J=8.2, ^{3}J=6.0, H-6a); 3.51 (dd, 1H, ^{2}J=15.5, ^{4}J=1.5, H-5a); 3.42 (d, 1H, ^{2}J=15.5, H-5b); 3.10 (ddd, 1H, ^{2}J=13.9, ^{3}J=7.9, ^{3}J=6.0, H-6b); 2.69 (ddd, 1H, ^{2}J=12.0, ^{3}J=8.4, ^{3}J=5.9, H-7a); 2.50-2.56 (m, 5H); 1.82-1.90 (m, 1H); 1.72 (br, 4H); 1.55-1.65 (m, 1H); 1.25-1.31 (m, 4H); 0.84-0.87 (m, 3H). ^{13}C NMR δ ($CDCl_3$, 100MHz, ppm, J_{C-F} = Hz); 171.1 (C-4); 61.9 (C-2); 54.1 (2C); 53.0 (C-7); 41.4 (C-6); 35.3; 32.0 (C-5); 26.2; 23.4 (2C); 22.3; 14.0; MS (70 eV): m/z (%) = 256 (M^+, 1); 252 (M - 4, 2); 102 (1); 97 (9); 84 (100); 69 (3).

2-phenyl-3-(2-(pyrrolidin-1-yl)ethyl)thiazolidin-4-one **4Bc**.

Oil; ^{1}H NMR δ ($CDCl_3$, 400MHz, ppm, J_{H-H} = Hz); 7.27-7.33 (m, 3H, Ar); 7.20-7.24 (m, 2H, Ar); 5.78 (d, 1H, ^{4}J=1.6, H-2); 3.74 (dt, 1H, ^{2}J=13.7, ^{3}J=6.7, H-6a); 3.72 (dd, 1H, ^{2}J=15.5, ^{4}J=1.9, H-5a); 3.64 (d, 1H, ^{2}J=15.4, H-5b); 2.72-2.79 (m, 2H, H-6b, H-7a); 2.61 (dt, 1H, ^{2}J=13.5, ^{3}J=6.8, H-7b); 2.37-2.40 (m, 4H); 1.65-1.69 (m, 4H). ^{13}C NMR δ ($CDCl_3$, 100MHz, ppm, J_{C-F} = Hz); 171.3 (C-4); 139.6 (Ar); 129.1 (Ar); 129.0 (2C, Ar); 127.0 (2C, Ar); 64.0 (C-2); 54.1 (2C); 53.1 (C-7); 41.6 (C-6); 32.9 (C-5); 23.4 (2C); MS (70 eV): m/z (%) = 276 (M^+, 1); 272 (M-4, 2); 178 (0.5); 135 (2); 121 (3); 97 (10); 84 (100); 70 (3).

3-(3-(diethylamino)propyl)-2-(4-fluorophenyl)thiazolidin-4-one **4Ca**.

Oil; ^{1}H NMR δ ($CDCl_3$), 600MHz, ppm, J_{H-H} = Hz); 7.35 (dd, 2H, ^{3}J=8.6, ^{4}J=5.2, Ar); 7.08 (t, 2H, ^{3}J=8.5, Ar); 5.75 (s, 1H, H-2); 3.79 (dd, 1H, ^{2}J=15.6, ^{4}J=1.5, H-5a); 3.72 (d, 1H, ^{2}J=15.6, H-5b); 3.62 (dt, 1H, ^{2}J=14.2, ^{3}J=7.6, H-6a); 2.78 (q, 4H, ^{3}J=7.2); 2.74-2.76 (m, 1H, H-6b); 2.65-2.70 (m, 1H, H-8a); 2.59-2.63 (m, 1H, H-8b); 1.75-1.82 (m, 2H, H-7); 1.12 (t, 6H, ^{3}J=7.2). ^{13}C NMR δ ($CDCl_3$, 150MHz, ppm, J_{C-F} = Hz); 171.5 (C-4); 163.0 (d, ^{1}J=248.8, Ar); 134.9 (d, ^{4}J=3.1, Ar); 129.2 (d, 2C, ^{3}J=8.4, Ar); 116.1 (d, 2C, ^{2}J=21.9, Ar); 62.7 (C-2); 49.1 (C-8); 45.6 (2C); 40.6 (C-6); 32.9 (C-5); 22.7 (C-7); 9.3 (2C). MS (70 eV): m/z (%) = 310 (M^+, 0.5); 281 (2); 182 (1); 109 (10); 86 (100); 72 (10).

2-(4-fluorophenyl)-3-(3-(piperidin-1-yl)propyl)thiazolidin-4-one **4Da**.

Oil; ^{1}H NMR δ ($CDCl_3$, 500MHz, ppm, J_{H-H} = Hz); 7.23 (dd, 2H, ^{3}J=8.6, ^{4}J=5.1, Ar); 7.00 (t, 2H, ^{3}J=8.5, Ar); 5.64 (d, 1H, ^{4}J=1.7, H-2); 3.72 (dd, 1H, ^{2}J=15.6, ^{4}J=1.8, H-5a); 3.62 (d, 1H, ^{2}J=15.6, H-5b); 3.57 (ddd, 1H, ^{2}J=14.1, ^{3}J=8.0, ^{3}J=6.6, H-6a); 2.63 (ddd, 1H, ^{2}J=13.9, ^{3}J=8.0, ^{3}J=5.9, H-6b); 2.18-2.23 (m, 5H, H-8a); 2.10-2.16 (m, 1H, H-8b); 1.60-1.66 (m, 1H, H-7a); 1.52-1.57 (m, 1H, H-7b); 1.45-1.49 (m, 4H); 1.34 (sl, 2H). ^{13}C NMR δ ($CDCl_3$, 125MHz, ppm, J_{C-F} = Hz); 171.0 (C-4); 162.9 (d, ^{1}J=248,0, Ar); 135.3 (d, ^{4}J=6.4, Ar); 128.9 (d, 2C, ^{3}J=8.2, Ar); 115.9 (d, 2C, ^{2}J=21.8, Ar); 62.9 (C-2); 56.0 (C-8); 54.3 (2C); 41.2 (C-6); 32.8 (C-5); 25.7 (2C); 24.2; 23.9 (C-7). MS (70 eV): m/z (%) = 322 (M^+, 2); 238 (1); 182 (1.5); 127 (4); 112 (4.5); 98 (100); 84 (9).

General procedure for the synthesis of hydrochloride salt **5Aa-f, 5Ba-d** and **5Ca-Ga**.

In a solution of thiazolidinone in dichloromethane (20 ml), was flowing the hydrochloric acid (HClg) (generate for the reaction of H_2SO_4 concentrated with NaCl) in a open vessel at room temperature for 30 minutes. The hydrochloride salt was filtered off under vacuum. When necessary, the salt was extracted with distilled water from the organic layer.

4-(2-(piperidin-1-yl)ethyl)-1-thia-4-azaspiro[4.5]decan-3-one chlorhydrate **5Ad**.

Brown solid; ^{1}H NMR δ (D_2O, 400MHz, ppm, J_{H-H} = Hz); 3.59 (t, 2H, ^{3}J=6.8, H-6); 3.48-3.52 (m, 2H); 3.48 (s, 2H, H-5); 3.12 (t, 2H, ^{3}J=6.8, H-7); 2.83 (dt, 2H, ^{2}J=12.4, ^{4}J=2.3); 1.74-1.83 (m, 4H); 1.63-1.70 (m, 5H); 1.28-1.60 (m, 6H); 0.93-1.04 (m, 1H); ^{13}C NMR δ (D_2O, 100MHz, ppm, J_{C-F} = Hz); 175.1 (C-4); 75.2 (C-2); 55.8 (C-7); 53.9 (2C); 37.1 (2C); 36.4 (C-6); 30.6 (C-5); 23.7; 23.0 (2C); 22.9 (2C); 21.0. MS (70 eV) (free base **4Ad**): m/z (%) = 171 (M-111, 0.5); 128 (2); 111 (6); 98 (100); 84 (2).

4-(2-(pyrrolidin-1-yl)ethyl)-1-thia-4-azaspiro[4.5]decan-3-one chlorhydrate **5Bd**.

Dark Brown solid; ^{1}H NMR δ (D_2O, 400MHz, ppm, J_{H-H} = Hz); 3.60-3.66 (m, 2H); 3.58 (t, 2H, ^{3}J=6.8, H-6); 3.49 (s, 2H, H-5); 3.25 (t, 2H, ^{3}J=6.7, H-7); 2.96-3.03 (m, 2H); 1.94-2.06 (m, 2H); 1.83-1.91 (m, 2H); 1.75-1.82 (m, 2H); 1.64-1.71 (m, 4H); 1.49 (d, 1H, ^{2}J=13.0); 1.32-1.44 (m, 2H); 0.93-1.05 (m, 1H). ^{13}C NMR δ (D_2O, 100MHz, ppm, J_{C-F} = Hz); 175.0 (C-4); 75.2 (C-2); 54.8 (2C, C-8); 54.0 (C-7); 37.8 (C-6); 37.0 (2C); 30.6 (C-5); 23.7; 23.1 (2C); 22.6 (2C). MS (70 eV) (free base **4Bd**): m/z (%) = 264 (M-4, 3); 171 (1); 128 (1); 97 (15); 84 (100); 69 (3).

Table 1: Chemical parameters of 2,3-substituted-1,3-thiazolidin-4-one derivatives

Nº	Yield (%)	MF	MW (g/mol)	Log P*	Dose (µmol/kg)**
4Aa	74%	$C_{16}H_{21}FN_2OS$	308.41	2.72	289.9
4Ba	89%	$C_{15}H_{19}FN_2OS$	294.39	2.30	302.2
4Ca	22%	$C_{16}H_{23}FN_2OS$	310.43	2.77	288.2
4Da	52%	$C_{17}H_{23}FN_2OS$	322.44	2.83	278.6
4Ea	44%	$C_{15}H_{13}FN_2OS$	288.34	2.81	307.8
4Fa	59%	$C_{14}H_{11}FN_2OS$	274.31	3.03	321.7
4Ab	64%	$C_{14}H_{26}N_2OS$	270.43	2.31	325.8
4Ac	68%	$C_{16}H_{22}N_2OS$	290.42	2.56	305.9
4Ad	48%	$C_{15}H_{26}N_2OS$	282.44	2.33	313.5
4Ae	33%	$C_{16}H_{21}FN_3O_3S$	335.42	2.52	268.9
4Af	71%	$C_{17}H_{24}FN_2O_2S$	320.45	2.44	280.2
4Bb	62%	$C_{13}H_{24}N_2OS$	256.41	1.90	341.4
4Bc	66%	$C_{15}H_{20}N_2OS$	276.40	2.14	319.6
4Bd	15%	$C_{14}H_{24}N_2OS$	268.42	1.91	328.0

FM = molecular formula; MW = molecular weight.
*Log P calculated by software Chendraw® Ultra, version 8.0.3
Dose in µmol/Kg corresponding to 100 mg/Kg of hydrochloride salt (5**)

Animals

Experiments were performed using 60 to 90 day-old adult male (Swiss) mice. Animals were maintained in controlled environmental conditions (22±1 °C, 12/12 h light/dark cycle, relative humidity (45 – 55 %) and free access to food and water [15]. All experiments were based on precepts and ethical considerations, to investigate experimental pain in animals [16]. The project was approved by the University Ethics Committee, registered under the number (CEEA 2231).

Standard drugs and negative control

Sodium dipyrone (Novalgina®, Aventis Pharma Ltd) was diluted in water at two concentration 250 mg/Kg and 500 mg/Kg.

Tramadol hydrochloride (Tramal®, Pfizer Ltd) was diluted in water at two concentration 25 mg/Kg and 50 mg/Kg.

Saline solution (sodium chloride 0,9 %, Equiplex Ltd) was used as a negative control. Vehicle and drugs were administered intraperitoneally in volume of 0.1ml/10g body weight.

Hot plate test

The hot plate test is a standard model test used to determine antinociceptive efficacy of drugs with central activity through acute thermal stimulation [17, 18]. The apparatus used (Hot Plate, model EFF361, Insigth®) consisted of an aluminum plate that is evenly heated, and it is surrounded by a transparent acrylic rectangular cage, which keeps animals confined, where access is done only by a higher opening that allows researchers to remove and include animals.

Animals were randomized into groups of eight subjects and were habituated in a room for at least 30 minutes before the experiments [15]. 24 hours before each experiment, animals were weighed and accustomed to procedure, to avoid the occurrence of new-induced analgesia which could provide false results (SIEGFRIED *et al*., 1987) [17]. In habituation, the mice were exposed to the same conditions that would be subject on the day of the experiment, except to the derivatives treatment, such as the researcher manipulation, the injection (saline) and the mice insertion in the equipment. Lastly, the animals were observed, for a minute, for their pain

responses and then they were removed from the equipment. If positive for pain responses, the animals were disposed from the next stage (test).

In the day of the test, the mice were placed separately on the apparatus, and the latency time (in seconds) for the nociceptive response (jumping or hind paw licking) was measured with a manual chronometer in times of 30, 60 and 90 minutes after the saline injection (NaCl 0,9 %, 10 mL/Kg, *ip*), 2,3-substituted-1,3-thiazolidin-4-ones derivatives (100 mg/Kg, *ip*), dipyrone (250 and 500 mg/Kg, *ip*) or tramadol (25 and 250 mg/Kg, *ip*); it was settled the upper cut-off time of 50s, to avoid possible tissue damage in animals. The temperature established in the experiment was set to 50±0,1 ºC.

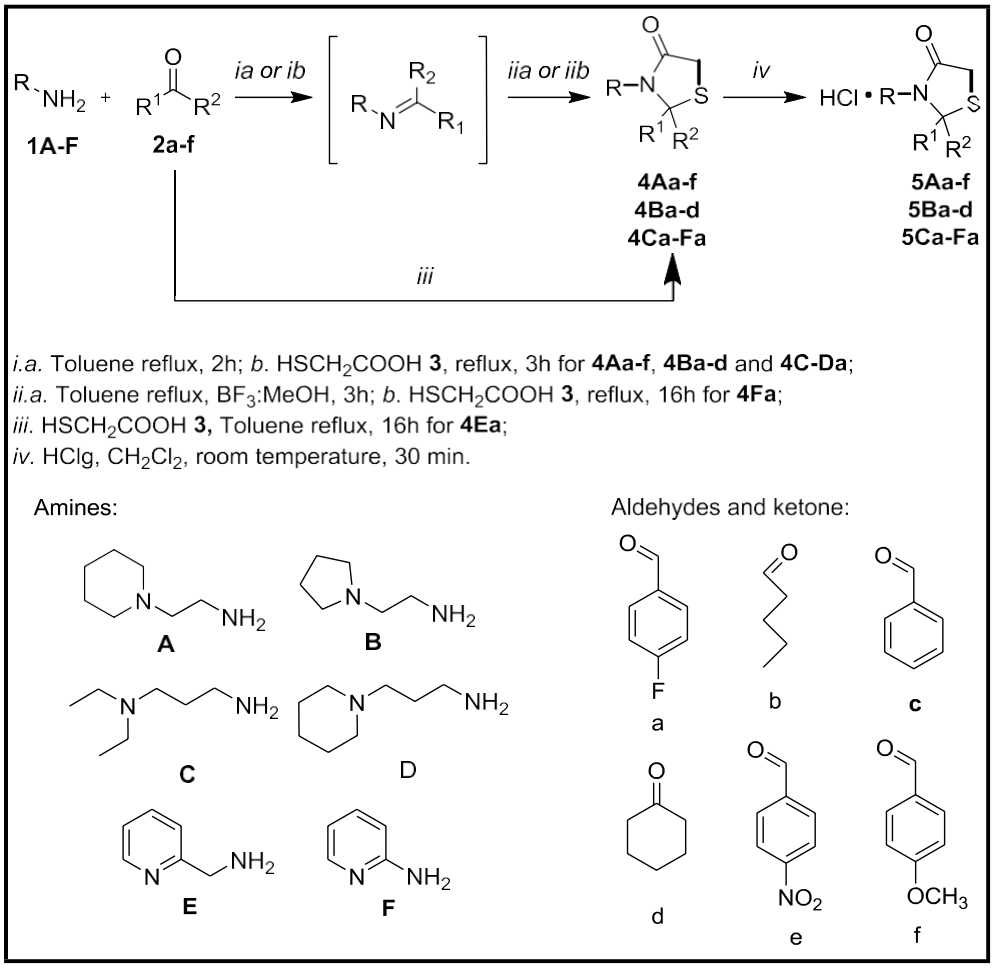
Finally, the animals were submitted to the hot plate test for three different moments after treatment administration, in 30, 60 and 90 minutes, and the latency time data were registered for further analysis [18, 20].

Statistical analysis

Data were analyzed by using variance analysis (ANOVA) followed by Duncan´s test in the SPSS 11.0.1 software. Furthermore, all data were expressed as mean ± standard error and the level of significance was set as $P< 0.05$.

3 Results and Discussion

Fourteen 1,3-thiazolidin-4-ones, eight of them unpublished in the literature, were synthesized to biological study. The new ones and the compounds **4Aa**, **4Ae**, **4Af**[14], **4Ea**, **4Fa**[13] and **4Ac** (WO 03\008398 A1, 2003) were synthesized from reaction between different amines **1A-F** with aldehyde or ketone **2a-f**in toluene refluxing with Dean-Stark apparatus for water removal by azeotropic distillation. The reaction conditions are demonstrated on Scheme 1 to obtain the compounds **4Aa-f, 4Ba-d, 4Ca-Fa**.



Scheme 1: Synthesis of proposals thiazolidinones and their hydrochloride salts.

The majority of products were obtained in moderate to good yields (Table 1). Lower yields for some products can be explained by the low reactivity of reactants like ketones, considering that ketones have a larger steric hindrance as compared with aldehydes by the presence of two substituents on the carbonyl carbon and by column chromatography purification process.

In sequence, the thiazolidinones passed for reaction to form a hydrochloride salt (**5**) in a basic nitrogen that all amines have in their structures. The salt allow water solubilization for injection, so no surfactant agent was needed for the application in animal model.

Efficient methodology was verified in these synthesis in view of the obtaining all the proposed products without observe the subproduct formation in any analyses. The structure of published thiazolidinones were confirmed by mass spectrometry (GC-MS). The new compounds were identified and characterized by mass spectrometry (GC-MS) and nuclear magnetic resonance (NMR) of ^{1}H and ^{13}C. The

NMR of **4Ab**, **4Ba**, **4Bb**,**4Bc**, **4Ca** and **4Da** were performed for the free base and the **5Ad** and **5Bd** were performed for the hydrochloride salt.

Then, the hydrochloride salts **5**were taken to test in animal model. On the hot plate test, pain response is described as latency time (in seconds), thus the high latency times indicate good inhibition of nociception. The latency times of 2,3-substituted-1,3-thiazolidin-4-ones on the hot plate test are shown in figures 1 to 6. Generally, the findings are according with others studies in the area, evidencing, once again, that 1,3-tiazolidin-4-ones are the important scaffold associated with many pharmacological activity [11]. Under these circumstances, from fourteen compounds tested, nine showed significant increases in latency time when compared to saline (negative control).

The antinociceptive activity of 1,3-tiazolidin-4-ones might be associated with their capacity to inhibit cyclooxygenase (COX) enzymes including COX-2 selective inhibition, according to many studies [21-24]. Enzyme´s inhibition shows antiinflammatory, analgesic and antipyretic activities due to the reduce of prostaglandins synthesis [25]. In this way, a preview study showed analgesic and antiinflammatory activities of 2-(aryl)-3-(4-sulfonamidebenzene)-1,3-thiazolidin-4-ones. This compound with 4-fluorophenyl, as aryl group, showed considerable COX-2 inhibition compared to NSAID nimesulide, a known COX-2 selective inhibitor [21]. In this sense, this research began studying 2-(4-fluorophenyl)-thiazolidinones with different amine cores linked at 3-position of the thiazolidinone ring (scheme 1).

In figure 2, it exhibits the effects of compounds **5A-Ga** on latency time 30 minutes after injection. Four compounds (**5Aa**, **5Ba**, **5Ea** and **5Fa**), by ANOVA/DUNCAN, revealed an increase in latency to nociceptive response when compared to saline ($P<0.05$), demonstrating that these compounds are capable to promote antinociceptive activity. However, no one was better than dipyrone or tramadol, two well-known analgesic medication, which act by different mechanisms of action. These standard drugs were selected, with positive controls, due to their strong analgesic effect, low-cost and larger current therapeutic use [26-28]. Although, the morphine is the standard drug to evaluate analgesic medication [18], it was chosen tramadol hydrochloride, due to its large used to relief acute or chronical pain of moderate or severe intensity [26, 27]. Nevertheless, to maintain the same analgesic effect, it was converted morphine (mg) into tramadol (mg), using tables of

equianalgesic opioid doses in parenteral administration (1 mg morphine = 10 mg de tramadol) [1, 29].

The difference in increases of latency time among **5Aa**, **5Ba**, **5Ea** and **5Fa** and dipyrone possibly occurred due to the dose used. Sodium dipyrone (333.3 g/mol; 250 mg/kg; 750 µmol/Kg; *ip*) had molar mass similar to 3-(amino)-2-(4-fluorophenyl)-1,3-thiazolidin-4-one derivatives (310,81 to 358,94 g/mol; 100 mg/kg; 323,7 to 278,6 µmol/Kg; *ip*), so there were more dipyrone moles available to act in nociceptive process. The dose of 100 mg/kg was based in previous [20, 22].

In the 60 and 90 minutes evaluation (Figure3 and 4), there were decreases in latency times, which were expected, while the compound **5Ea**,still maintained higher levels of antinociceptive activity than saline. Thus, this suggests that those tested compounds have a short effect and fast metabolization, but it is necessary others studies to delineate and confirm this hypothesis. Yet, a previous study investigated antiinflammatory and analgesic activity of 3,3′-(1,2-ethanediyl)-bis[2-aryl-4-thiazolidinone], obtaining good results and latency times in hot plate test as phenylbutazone and indomethacin did, in up to 180 minutes after treatment. These compounds had two 1,3-thiazolidin-4-one nucleus, connected by an ethyl (position 3), and there are two substituted phenyl group (position 2) [20].

Based on the results showed on figure 2,assessment of 30 minutes, were chosen to the sequence of study, in order to plan new structural modification, the two compounds with greater intensity effect, expressed byhigher latency times:2-(4-fluorophenyl)-3-(piperidin-1-ylethyl)-1,3-thiazolidin-4-one (**5Aa**; 24,3±5,6s);and 2-(4-fluorophenyl)-3-(pyrrolidin-1-ylethyl)-1,3-thiazolidin-4-one (**5Ba**; 20,3±2,6s). Although, the compound **5Ea** has retained the antinociceptive effect before 60 and 90 minutesit was considered the structural similarity between **5Aa** and **5Ba** allowing a improve study and comprehension of structure-activity relationship of 4-thizolidinones.

Thereby, a second series of 1,3-thiazolidin-4-ones (Scheme 1) was obtained. For this, the amines **A** (2-(piperidin-1-yl)ethan-1-amine) and **B** (2-(pyrrolidin-1-yl)ethan-1-amine) were kept, and the halogenated aromatic aldehyde (4-fluorobenzaldehyde **a**) was modified in the synthetic process, obtaining compounds based on other carbonyl group (aldehydes and ketone) (Scheme 1). The results of those compounds are shown in tables 5, 6 and 7 in evaluation of 30, 60 and 90 minutes, respectively.

The compounds **5Ab**, **5Ac**, **5Ad**, **5Bb** and **5Bd,** through ANOVA/DUNCAN, revealed an increase in latency to nociceptive response when compared to saline ($P<0.05$) in the 30 minutes evaluation after treatment. Furthermore, even after the increase in the control dose, the compounds **5Ab**, **5Ac, 5Ad** showed latency to nociceptive response compared to dipyrone ($P<0.05$), but not equal or better than tramadol. Moreover, literature findings demonstrated that 1,3-thiazolidin-4-one derivatives have a similar antiinflammatory and analgesic efficacy to NSAIDs [19-21]. When evaluating these compounds in 60 and 90 minutes, none of them showed analgesic effects.

Higher latency times were obtained by 2-butyl-3-(2-piperidin-1-yl)ethyl)thiazolidin-4-one (**5Ab**; 32.4±3.5s), 2-phenyl-3-(2-piperidin-1-yl)ethyl)thiazolidin-4-one (**5Ac**; 27.8±4.3s) and 4-(2-piperidin-1-yl)ethyl)-1-thia-4-azaspiro[4.5]decan-3-one (**5Ad**; 31.6±4.3s). Therefore, these 2-substituents (**b**, **c**, **d**) apparently were associated a better antinociceptive activity then 4-fluorophenyl (**a**). Unsal-tan *et al* (2012), investigated 2,3,5-trisubstituted-1,3-thiazolidin-4-one (2-aryl-2,5,5-trimethyl-3-(4-methylsulfonylphenylamino)-1,3-thiazolidin-4-one) derivatives with a promising COX-2 selective inhibitory activity, modifying the substituent linked to sulfonyl group by NH_2 or CH_3, and position 4 of 2-aryl by H, Cl, F, CH_3 and CF_3. So, it was concluded that compounds bearing halogen atoms on the phenyl group ring (position 2) were non-selective, while compounds possessing methyl group exhibited highly COX-2 inhibitor selectivity and potency. However, it should be considered that methylsulfone and sulfonamide groups are characteristic of COX-2 selective inhibition [23].

In structure-activity relationship through compounds tested, a subtle change of 3-piperidin-1-ylethyl (**5Aa**; 24.3±5.6 s) to 3-pyrrolidin-1-ylethyl (**5Ba**; 20.3±2.6 s) does not seem to promote significant change when assess at 30 minutes and 4-fluorophenyl (**a)** is present. However, by replacing in position 3 for other substituents (**b**, **c** and **d**), 3-piperidin-1-ylethyl **A** is apparently better than 3-pyrrolidin-1-ylethyl**B**, in relation to latency time. Because of this, the hot plate test evaluates antinociceptive efficacy of drugs with central analgesic activity [17], so lipophilicity is important to access the site of action of these compounds.

Vigorita *et al* (2001) assessed analgesic activity of 3,3′-(1,2-ethanediyl)-bis[2-aryl-4-thiazolidinone] and obtained good latency times and overall results like phenylbutazone and indomethacin. Similarly, in our work, derivatives compound of

amines A and B also obtained good results. In this way, the ethyl group between the atoms of nitrogen (position 3) may seem important for the antinociceptive activity. The **5Da** which has propyl group did not show increases in latency time as **5Aa** did.

Another structural change which also did not result in a significant modification of latency time in 30 minutes was between 3-(2-pyridin-2-ylmethyl) (**5Ea**) and 3-(2-pyridin-2-yl) (**5Fa**). Thus, it demonstrated that the closeness of two or one carbons between the nitrogen atoms possibly does not promote interference in the activity. However, separating nitrogens of the ring and the 3-amino-substituent for three carbons atoms possibly promote decreases in antinociceptive activity, since 3-(piperidin-1-ylethyl) (**5Da**) and 3-(2-(diethylamino)ethyl) (**5Ca**) showed no significantly increases in latency time.

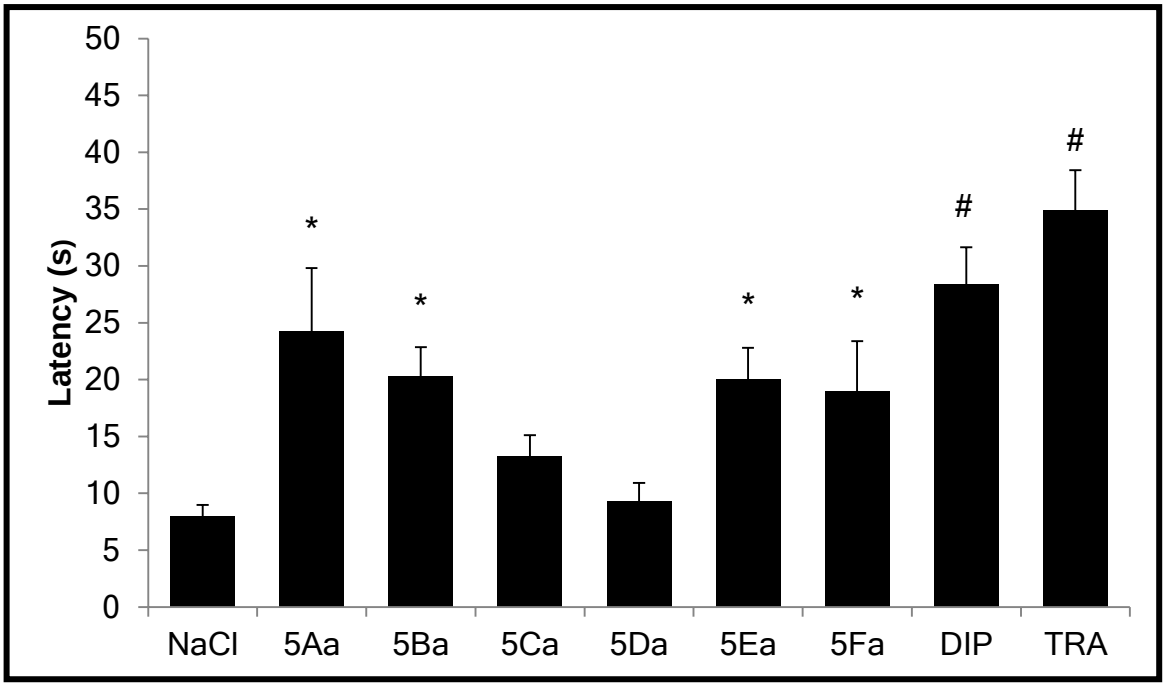


Figure 2: Latency time of ***3-amino-2-(4-fluorophenyl)-1,3-thiazolidin-4-one*** derivatives (**5A-Ga**) in hot plate test evaluation in ***30 min*** (100 mg/Kg, *ip*). DIP = Sodium dipyrone (250 mg/Kg); TRA = Tramadol hydrochloride (25 mg/Kg). *P< 0.05 X saline; # P<0.05 X negative control and compounds. ANOVA/DUNCAN.

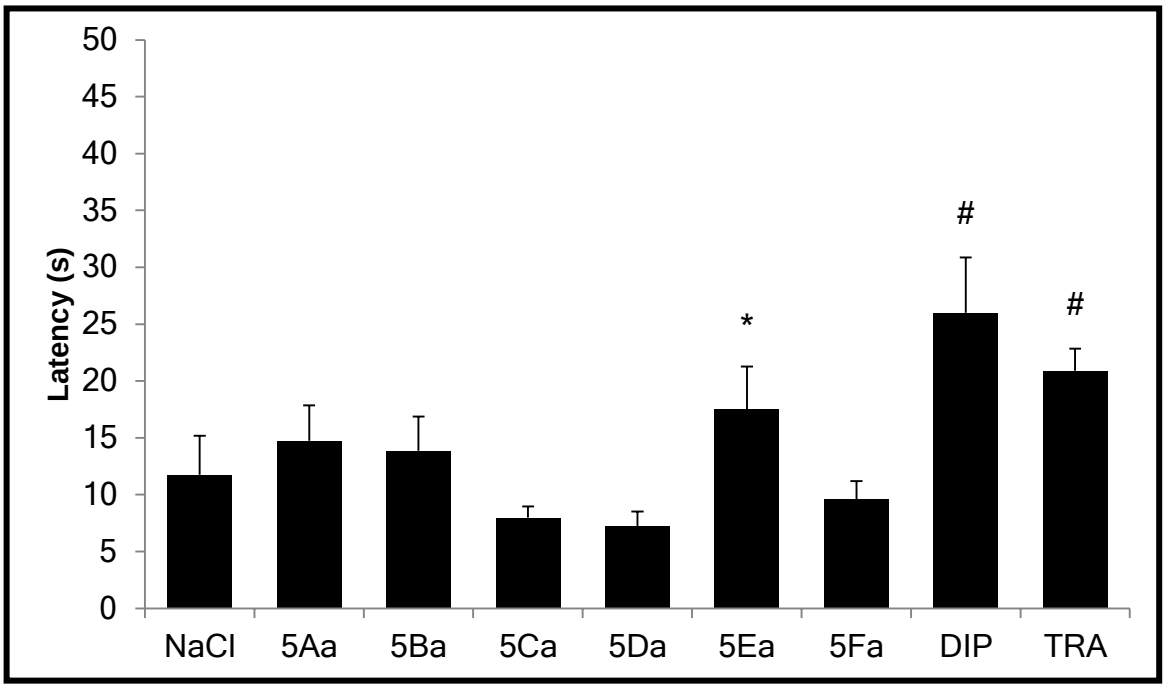


Figure 3: Latency time of ***3-amino-2-(4-fluorophenyl)-1,3-thiazolidin-4-one*** derivatives (**5A-Ga**) in hot plate test evaluation in ***60 min*** (100 mg/Kg, *ip*). DIP = Sodium dipyrone (250 mg/Kg); TRA = Tramadol hydrochloride (25mg/Kg). *P< 0.05 X saline; # P<0.05 X negative control and compounds. ANOVA/DUNCAN.

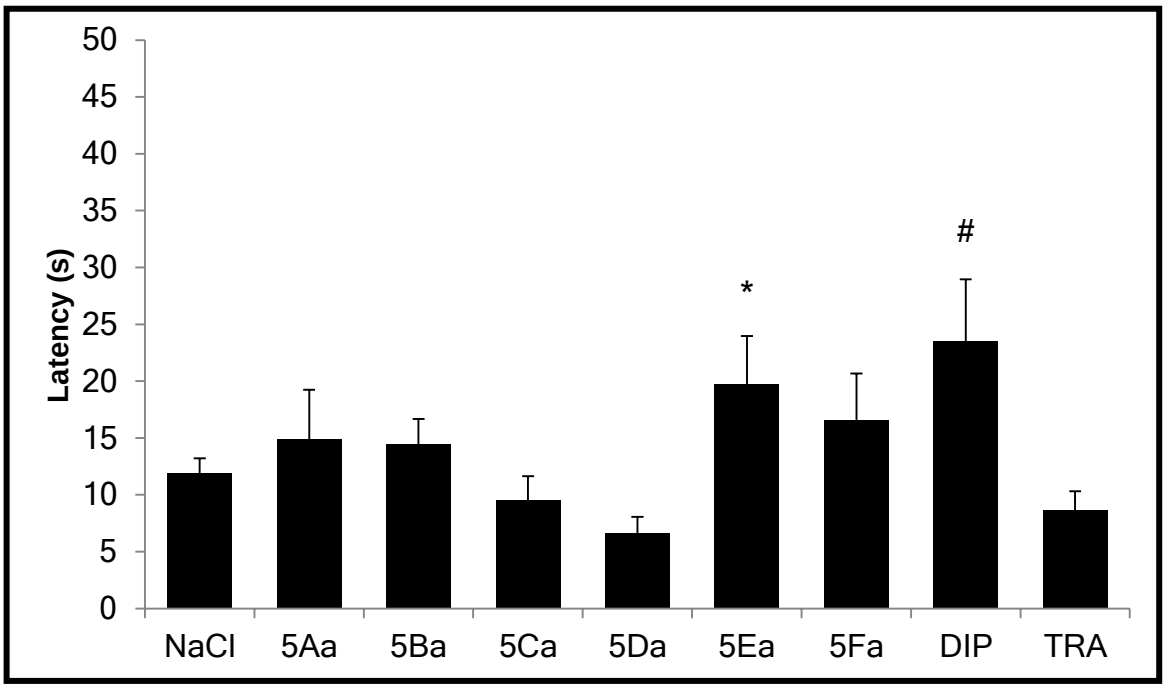


Figure 4: Latency time of ***3-amino-2-(4-fluorophenyl)-1,3-thiazolidin-4-one*** derivatives (**5A-Ga**) in hot plate test evaluation in ***90 min*** (100 mg/Kg, ip). DIP = Sodium dipyrone (250 mg/Kg); TRA = Tramadol hydrochloride (25mg/Kg). *P< 0.05 X saline; # P<0.05 X negative control and compounds. ANOVA/DUNCAN.

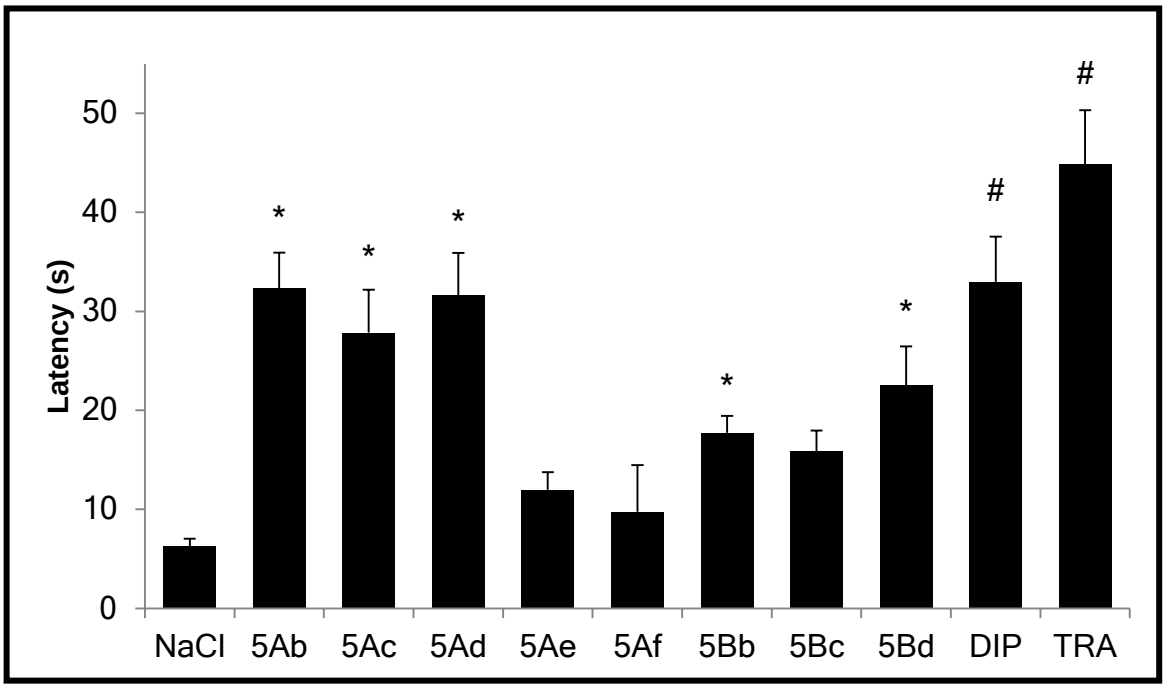


Figure 5: Latency time of ***3-(piperidin-1-il)-ethyl-2-aldeido(ketone)-1,3-thiazolidin-4-one*** derivatives (**5Ab-f**) ***3-(pyrrolidin-1-il)-ethyl-2-aldeido(ketone)-1,3-thiazolidin-4-one*** (**5Bb-d**) derivatives in hot plate test evaluation in ***30 min*** (100 mg/Kg, ip). DIP = Sodium dipyrone (500 mg/Kg); TRA = Tramadol hydrochloride (50 mg/Kg). *P< 0.05 X saline; # P<0.05 X negative control and compounds. ANOVA/DUNCAN.

4 Conclusions

This research described the antinociceptive activity of 1,3-thiazolidin-4-one derivatives through thermal stimulation in mice. The 1,3-thiazolidin-4-ones were synthesized from a multicomponent one-pot reaction including different **1A-F** amines with aldehyde or ketone **2a-f**.

These compounds were administered intraperitoneally in doses of 100 mg/Kg, using as control saline solution (NaCl 0,9%), sodium dipyrone (250 or 500 mg/Kg) and tramadol hydrochloride (25 or 50 mg/Kg). From the fourteen compounds tested, nine showed significant increases (**5Aa**, **5Ab**, **5Ac**, **5Ad**, **5Ba**, **5Bb**, **5Bd**, **5Ea**, **5Fa**) in latency time in the hot plate test in comparison to saline, and three (**5Ab**, **5Ac** and **5Ad**) showed latency to nociceptive response compared to sodium dipyrone (500mg/Kg) in evaluation of 30 minutes. The compound 2-(4-fluorophenyl)-3-(pyridin-2-ylmethyl)thiazolidin-4-one (**5Ea**) was the only that retained the antinociceptive effect in assessment of 30, 60 and 90 minutes.

In the structure-activity relationship, the highest latency times were obtained at the 3-(2-piperidin-1-yl)ethyl)thiazolidin-4-one derivatives (**5Ab** ,**5Ac** and **5Ad**), although other amines also showed promising results. Yet, substituents 2-butyl (**b**),

2-phenyl (**c**) and 2-cyclohexane (**d**) promote greater increases in the latency time than 4-fluorophenyl (**a**) when the amine was **A**.Moreover, the substitution of 4-nitrophenyl (**e**) or 4-methoxyphenyl (**f**) did not prove to be favorable for antinociceptive activity of these compounds.

Lastly, studies are needed to evaluate the antinociceptive activity of isomeric forms including COX-2. Then it is emphasized that a new study is being conducted to evaluate the analgesic activity of analogous **5Ea** and **5Fa**, as well as evaluation of anti-inflammatory and antipyretic activity of all compounds with significant results.

5 Acknowledgements

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Supplementary Material

The antinociceptive evaluation of 2,3-substituted-1,3-thiazolidin-4-ones through thermal stimulation in mice

Arthur Hipolito da Silva Neves
Daniel Schuch da Silva
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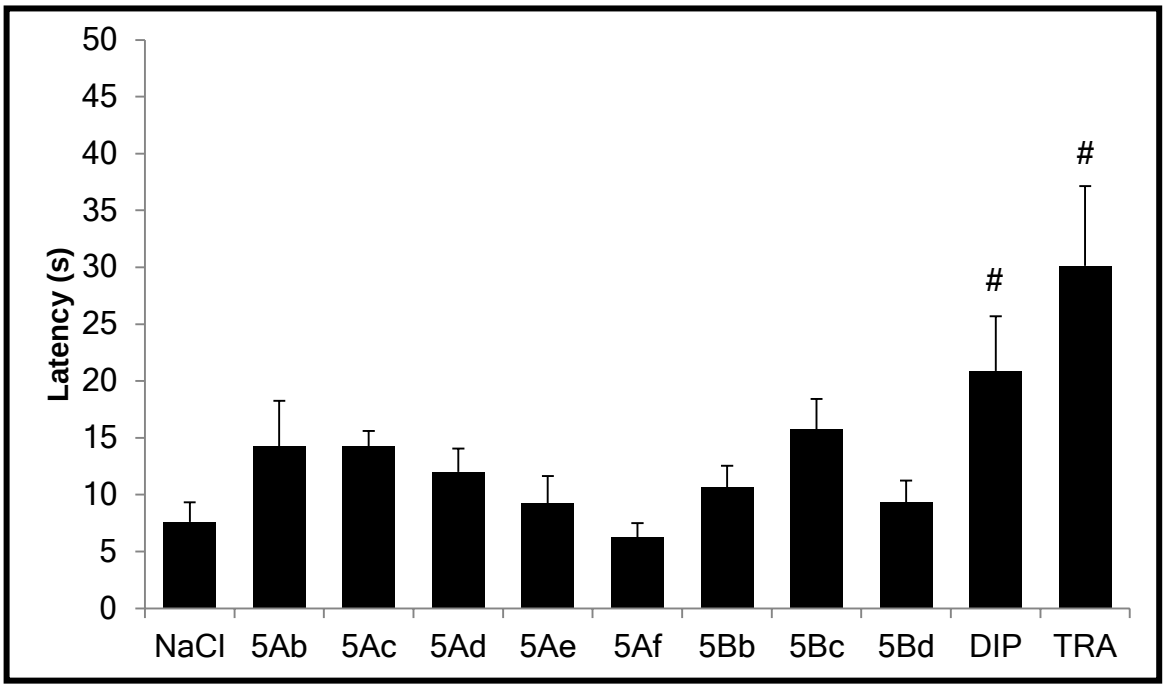

Figure S1: Latency time of ***3-(piperidin-1-il)-ethyl-2-aldeido(ketone)-1,3-thiazolidin-4-one*** derivatives (**5Ab-f**) ***3-(pyrrolidin-1-il)-ethyl-2-aldeido(ketone)-1,3-thiazolidin-4-one*** (**5Bb-d**) derivatives in hot plate test evaluation in ***60 min*** (100 mg/Kg, *ip*). DIP = Sodium dipyrone (500 mg/Kg); TRA = Tramadol hydrochloride (50 mg/Kg). *P< 0.05 X saline; # P<0.05 X negative control and compounds. ANOVA/DUNCAN.

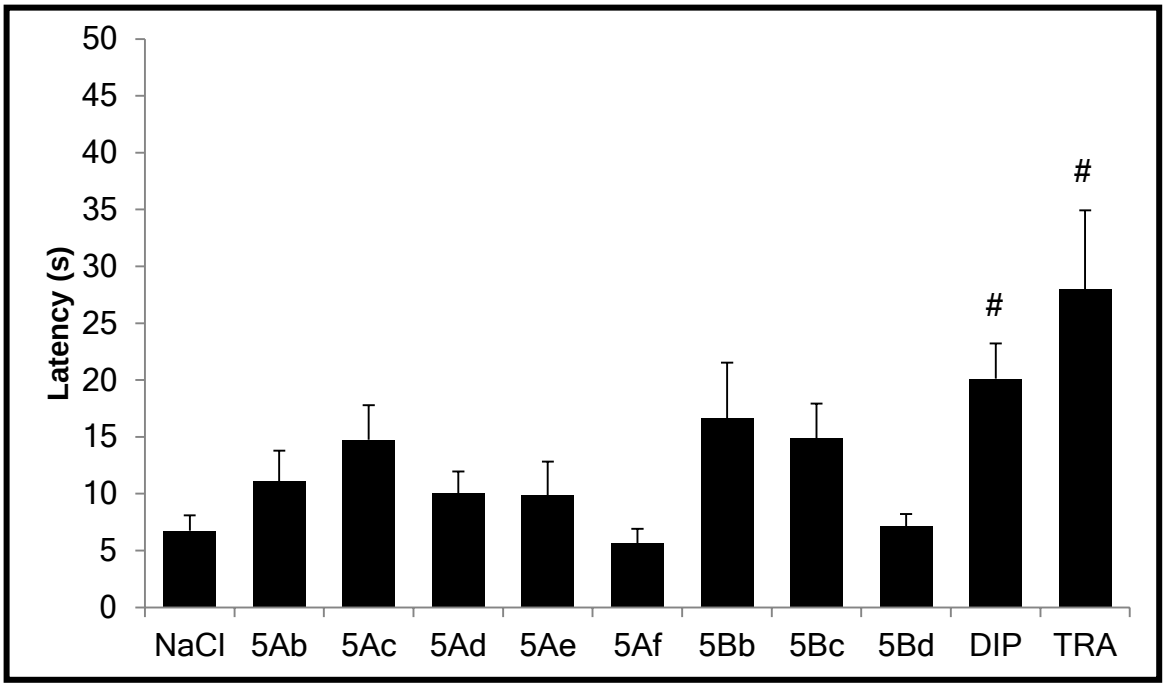

Figure S2: Latency time of ***3-(piperidin-1-il)-ethyl-2-aldeido(ketone)-1,3-thiazolidin-4-one*** derivatives (**5Ab-f**) ***3-(pyrrolidin-1-il)-ethyl-2-aldeido(ketone)-1,3-thiazolidin-4-one*** (**5Bb-d**) derivatives in hot plate test evaluation in ***90 min*** (100 mg/Kg, *ip*). DIP = Sodium dipyrone (500 mg/Kg); TRA = Tramadol hydrochloride (50 mg/Kg). *P< 0.05 X saline; # P<0.05 X negative control and compounds. ANOVA/DUNCAN.

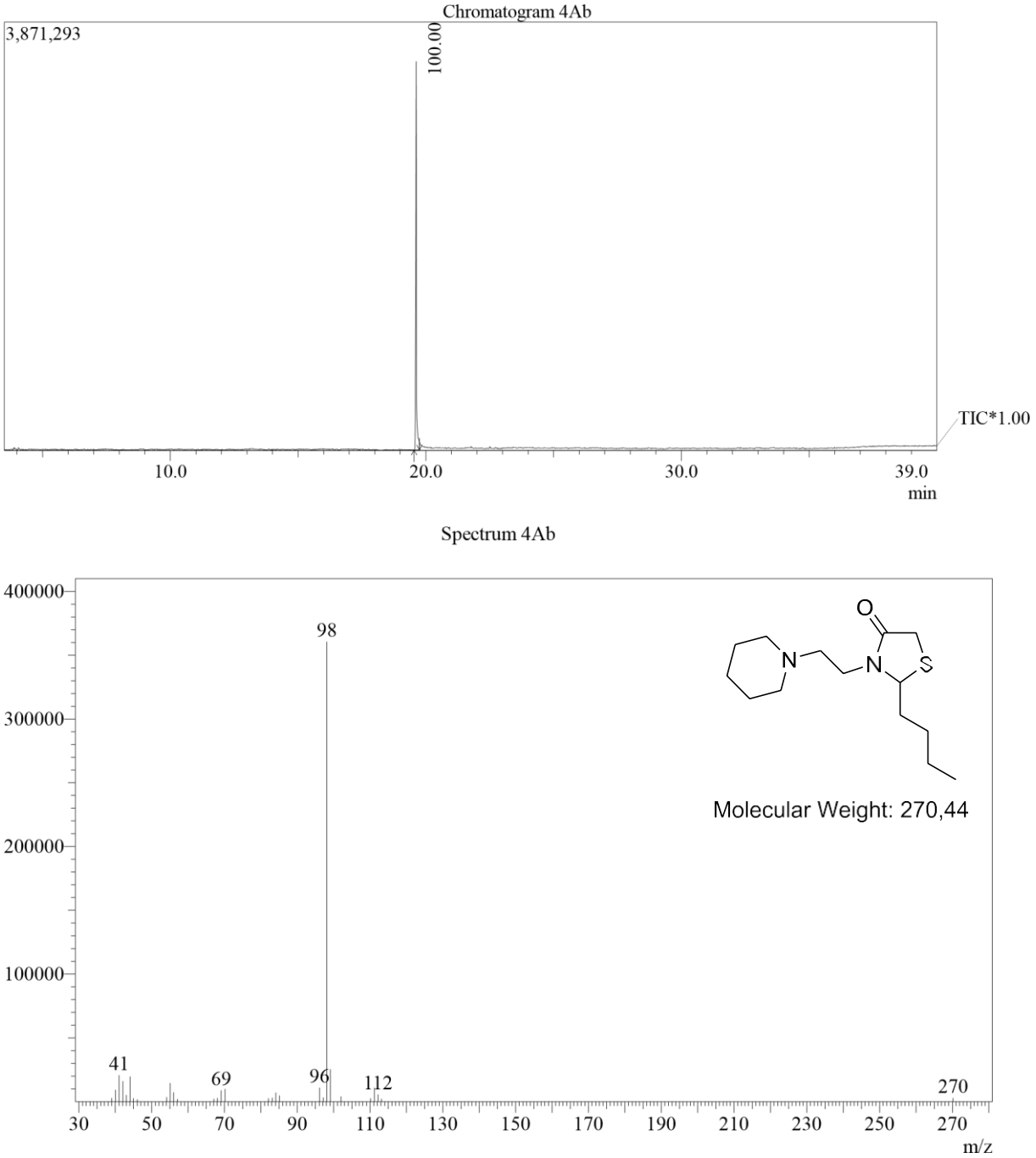

Figure S3: GC/MS of compound 2-butyl-3-(2-(piperidin-1-yl)ethyl)thiazolidin-4-one **4Ab**.

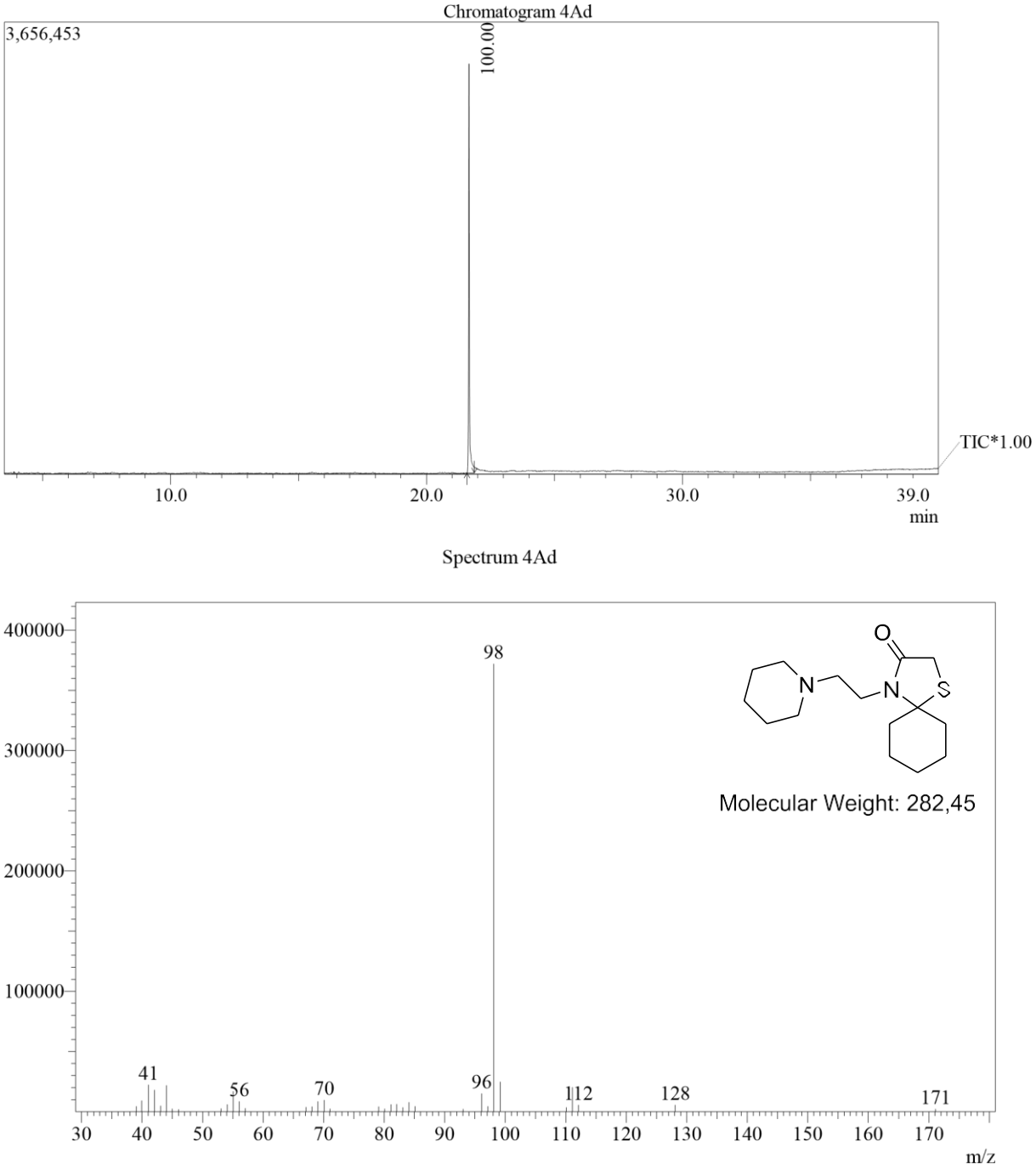

Figure S4: GC/MS of compound 4-(2-(piperidin-1-yl)ethyl)-1-thia-4-azaspiro[4.5]decan-3-one **4Ad**.

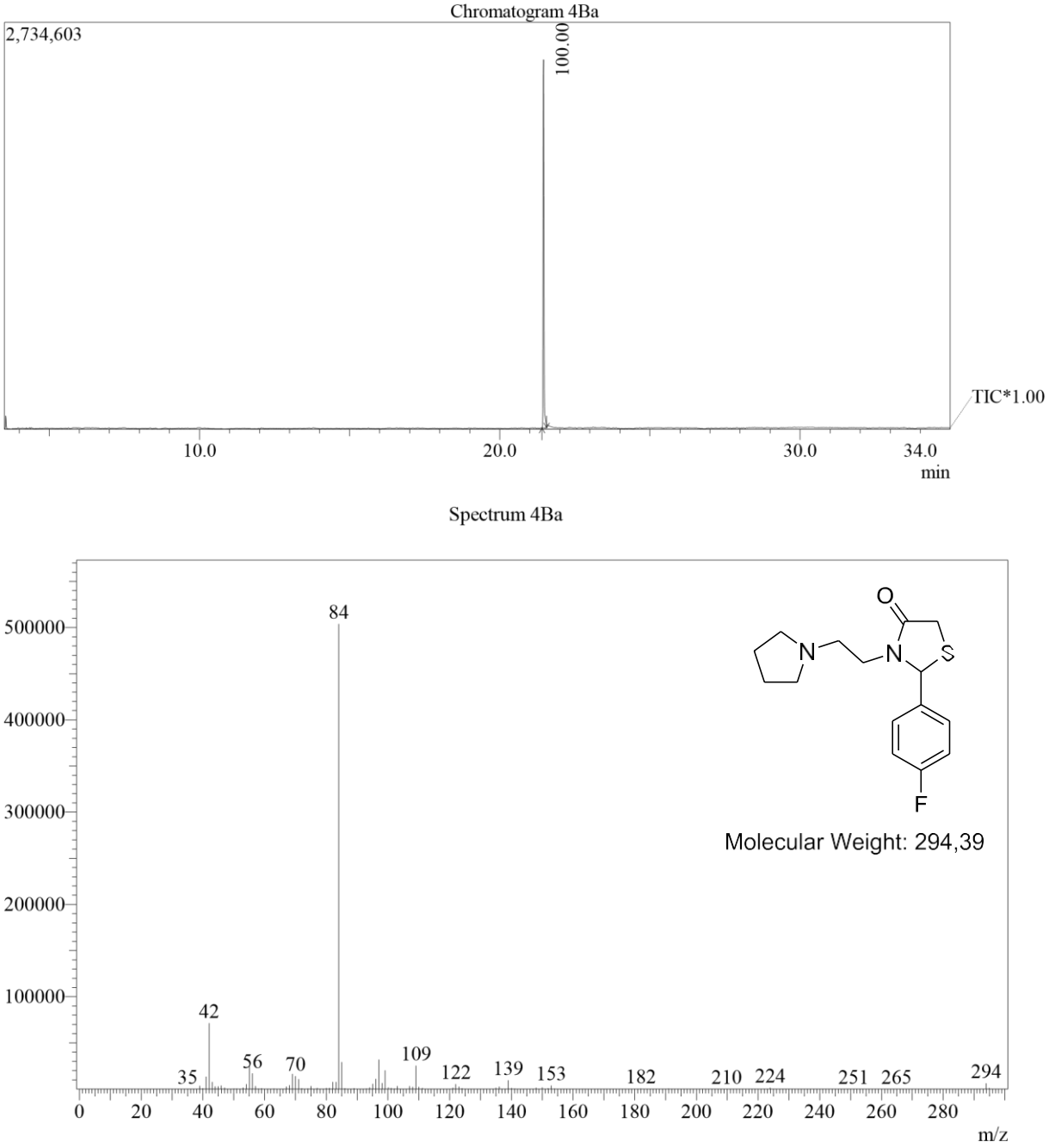

Figure S5: GC/MS of compound 2-(4-fluorophenyl)-3-(2-(pyrrolidin-1-yl)ethyl)thiazolidin-4-one **4Ba**.

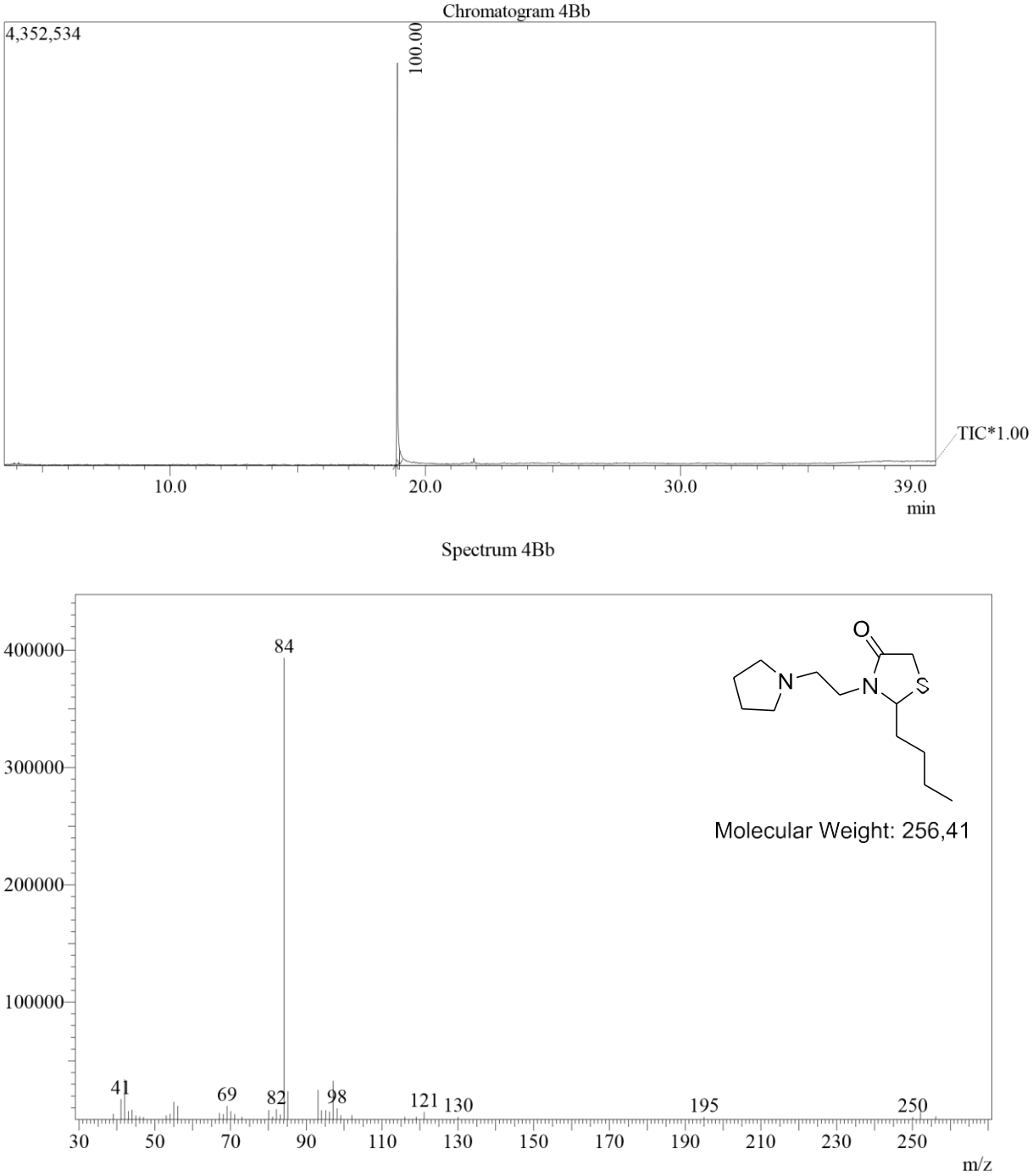

Figure S6: GC/MS of compound 2-butyl-3-(2-(pyrrolidin-1-yl)ethyl)thiazolidin-4-one **4Bb**.

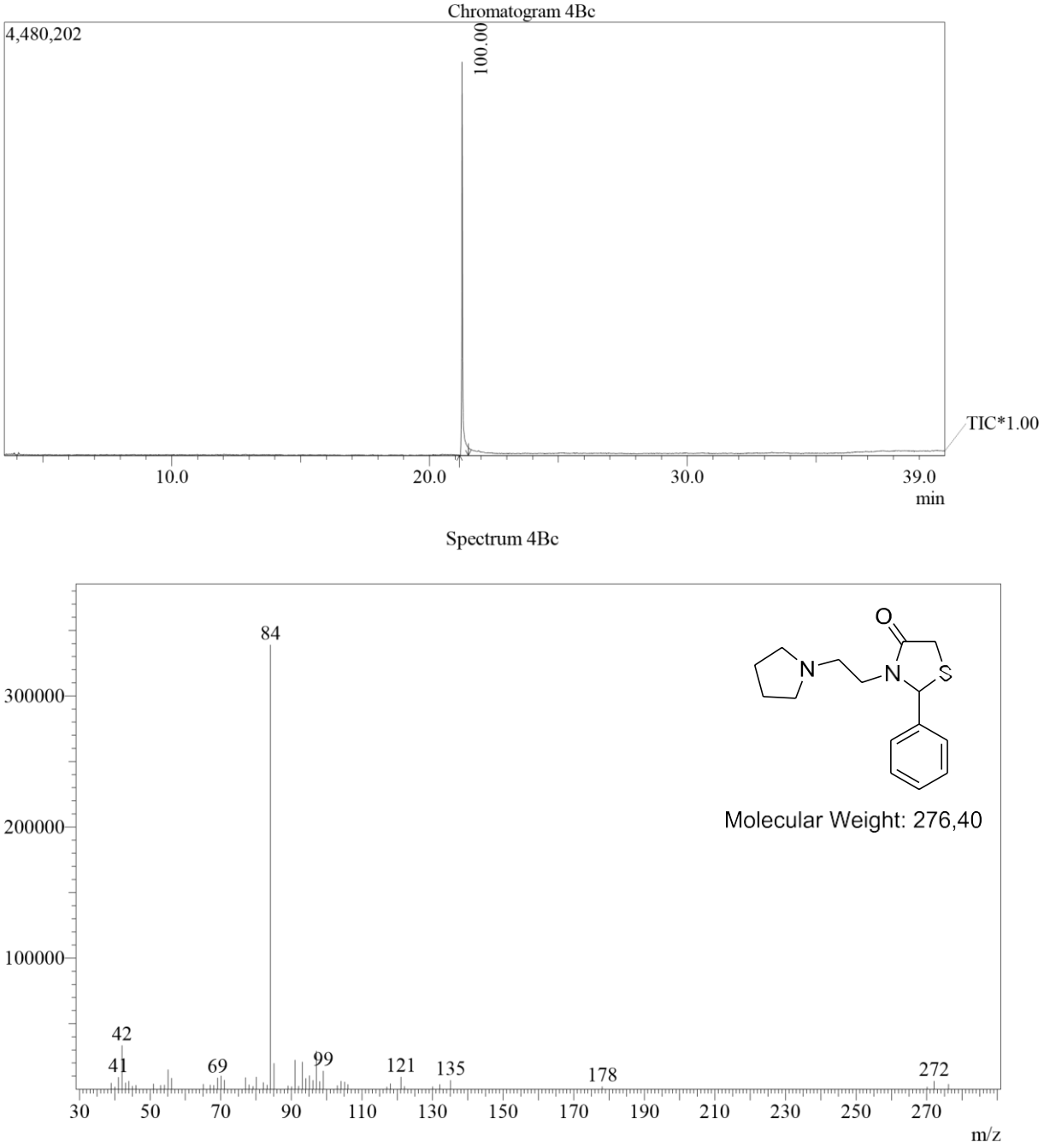

Figure S7: GC/MS of compound 2-phenyl-3-(2-(pyrrolidin-1-yl)ethyl)thiazolidin-4-one **4Bc**.

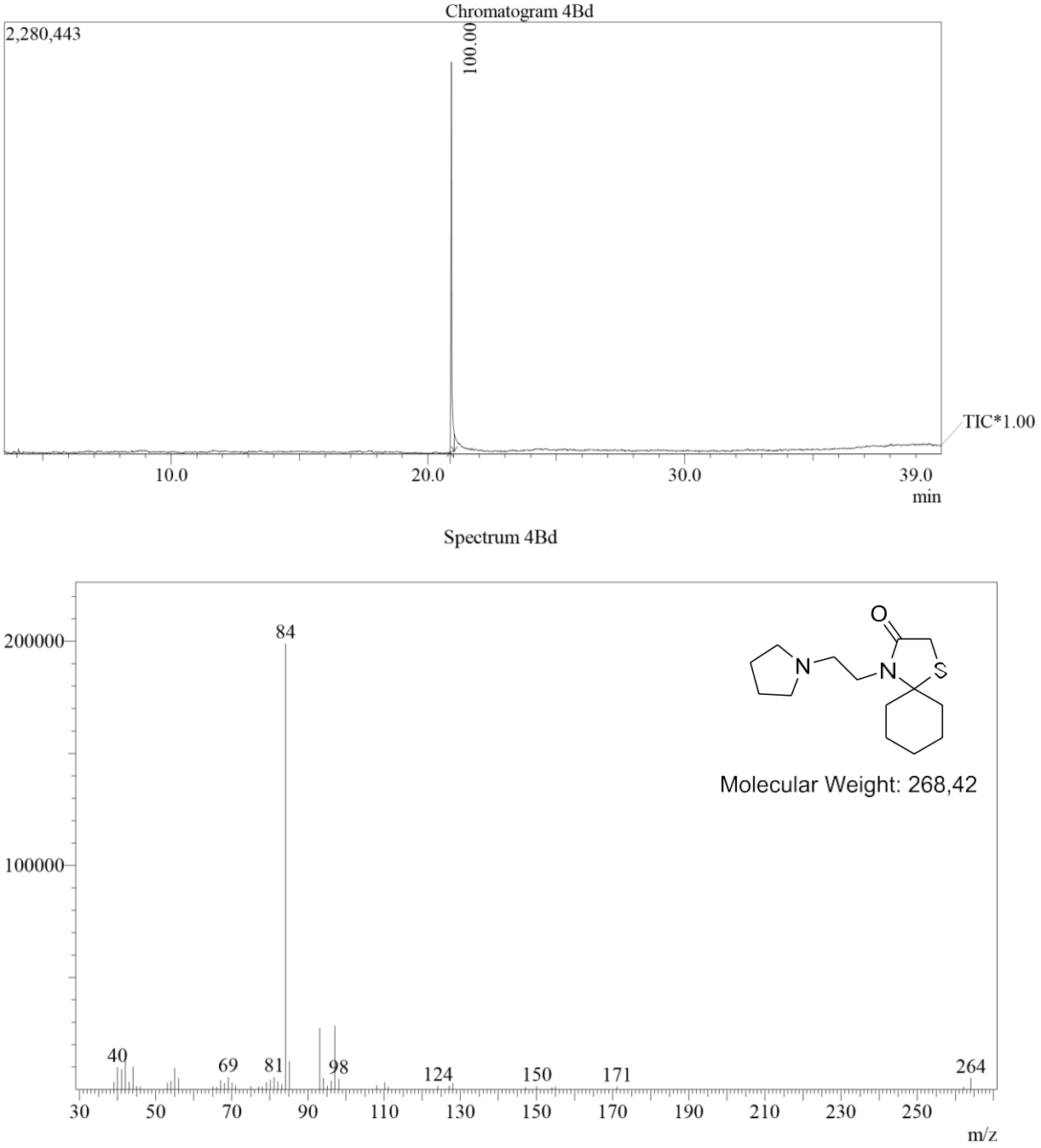

Figure S8: GC/MS of compound 4-(2-(pyrrolidin-1-yl)ethyl)-1-thia-4-azaspiro[4.5]decan-3-one **4Bd**.

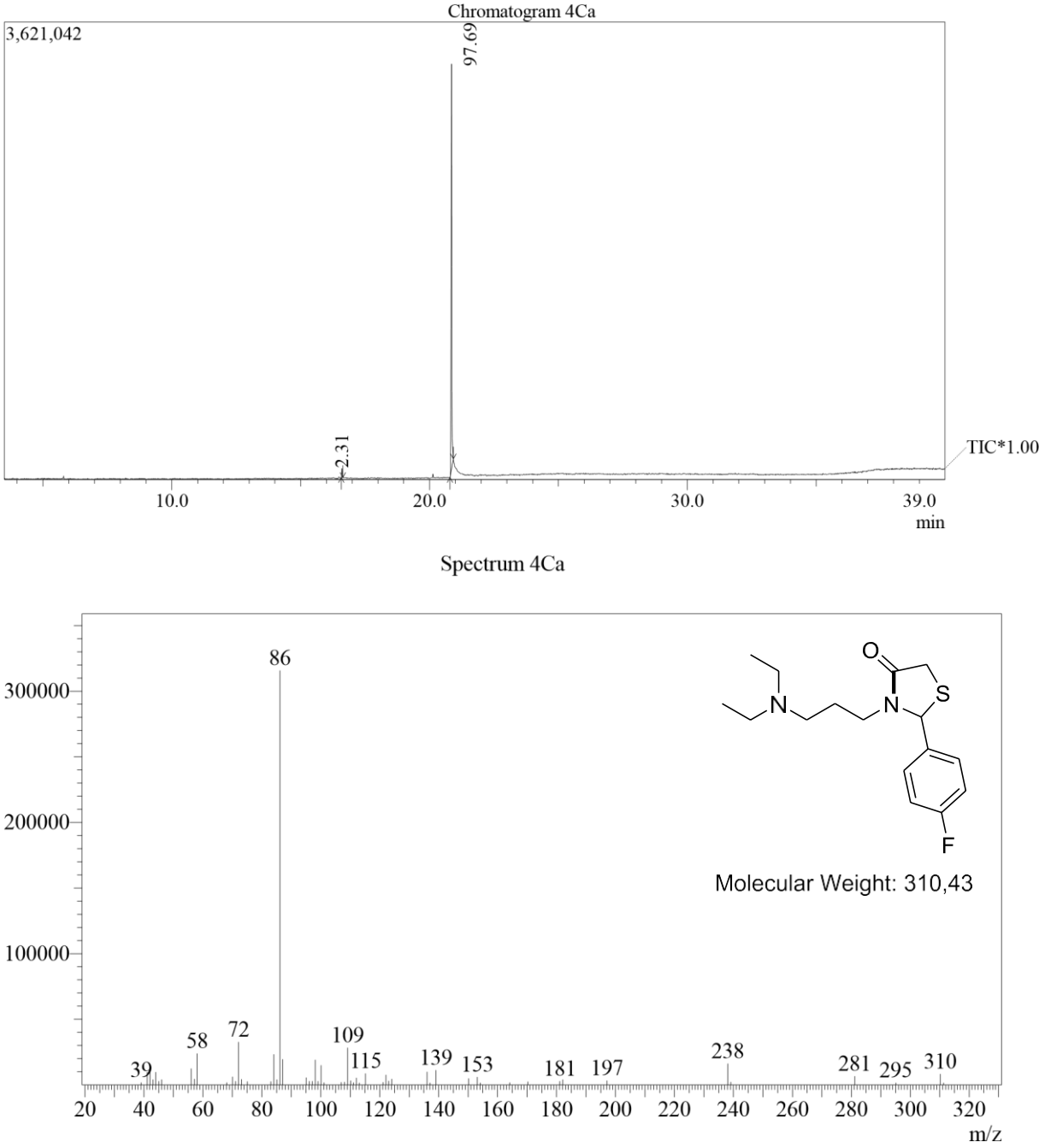

Figure S9: GC/MS of compound 3-(3-(diethylamino)propyl)-2-(4-fluorophenyl)thiazolidin-4-one **4Ca**.

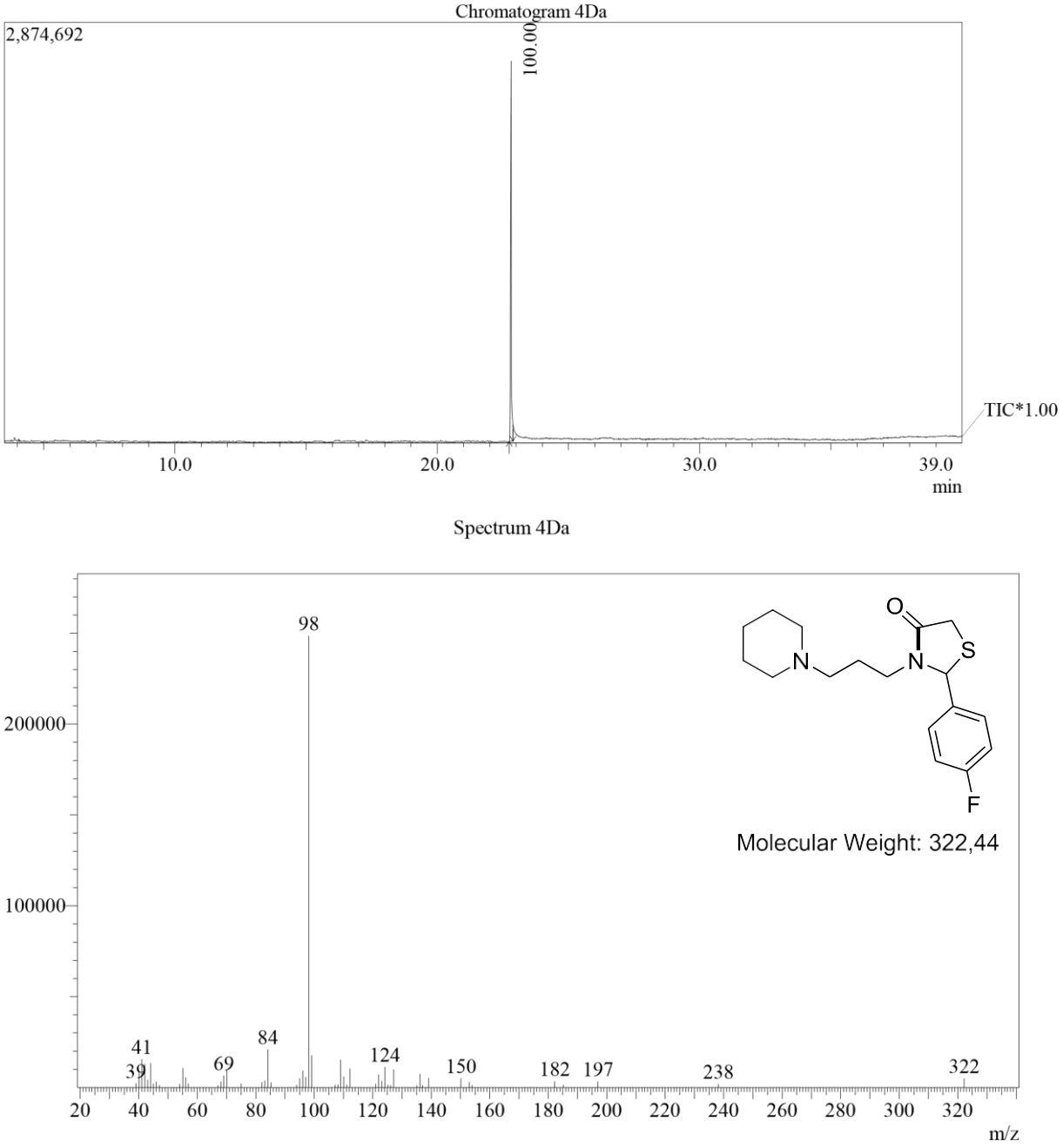

Figure S10: GC/MS of compound 2-(4-fluorophenyl)-3-(3-(piperidin-1-yl)propyl)thiazolidin-4-one **4Da**.

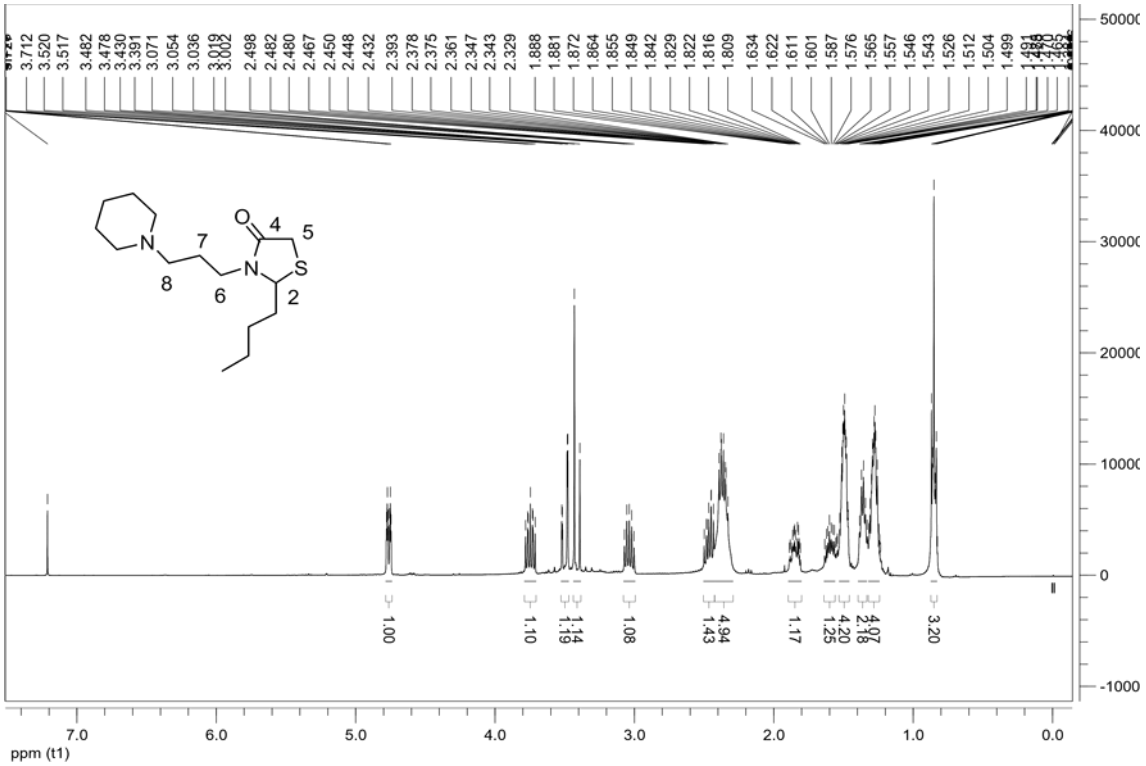

Figure S11: ^{1}H NMR spectrum of compound 2-butyl-3-(2-(piperidin-1-yl)ethyl)thiazolidin-4-one **4Ab**.

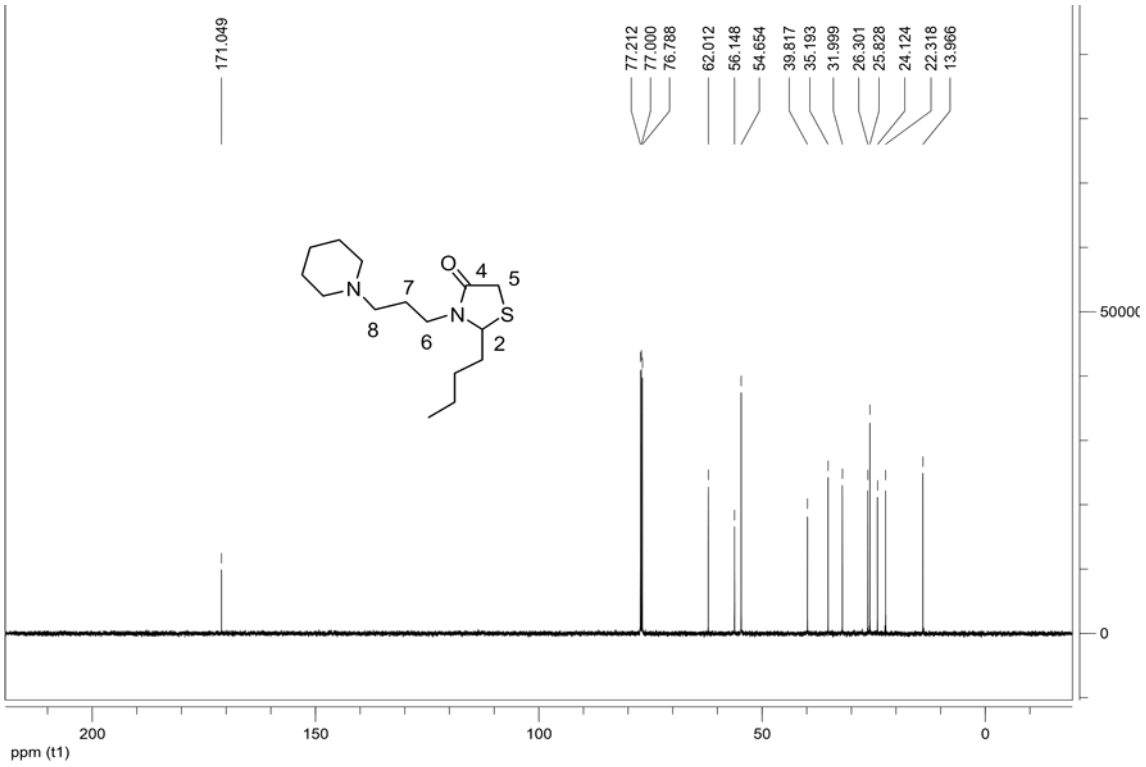

Figure S12: ^{13}C NMR spectrum of compound 2-butyl-3-(2-(piperidin-1-yl)ethyl)thiazolidin-4-one **4Ab**.

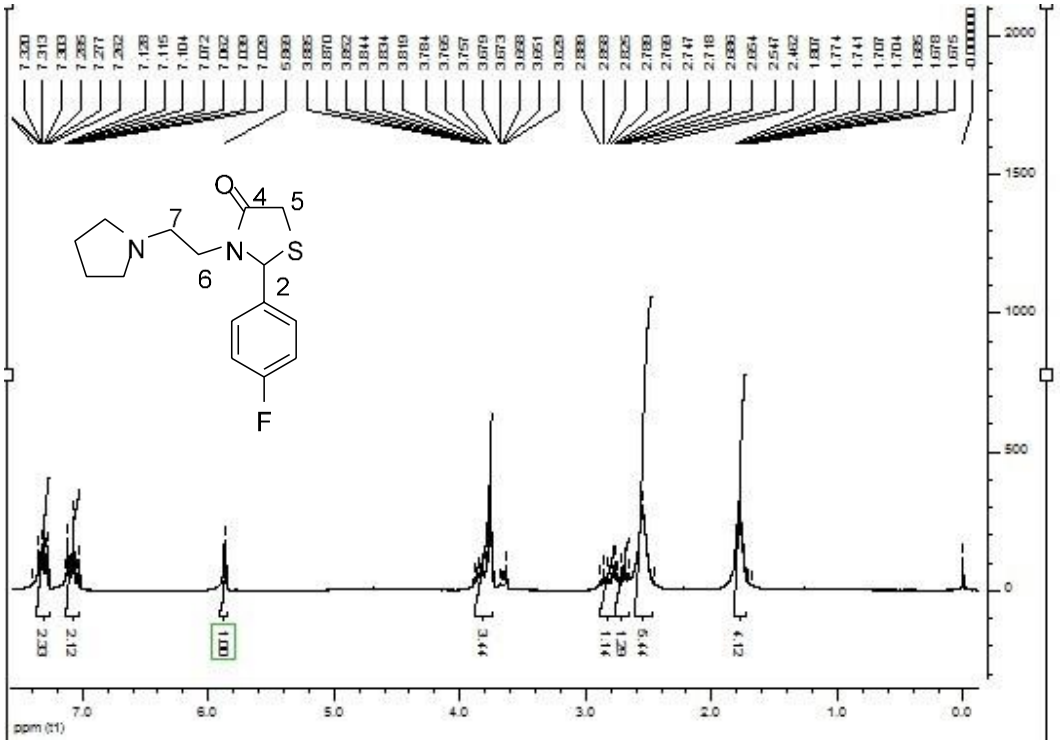

Figure S13: ^{1}H NMR spectrum of compound 2-(4-fluorophenyl)-3-(2-(pyrrolidin-1-yl)ethyl)thiazolidin-4-one **4Ba**.

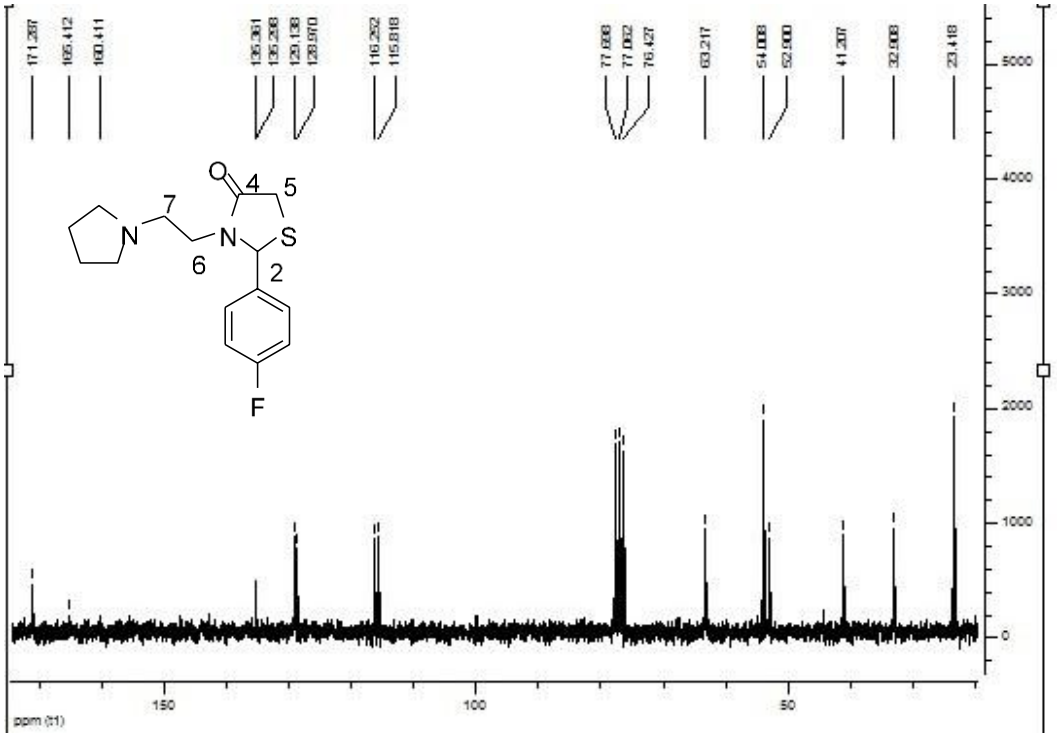

Figure S14: ^{13}C NMR spectrum of compound 2-(4-fluorophenyl)-3-(2-(pyrrolidin-1-yl)ethyl)thiazolidin-4-one **4Ba**.

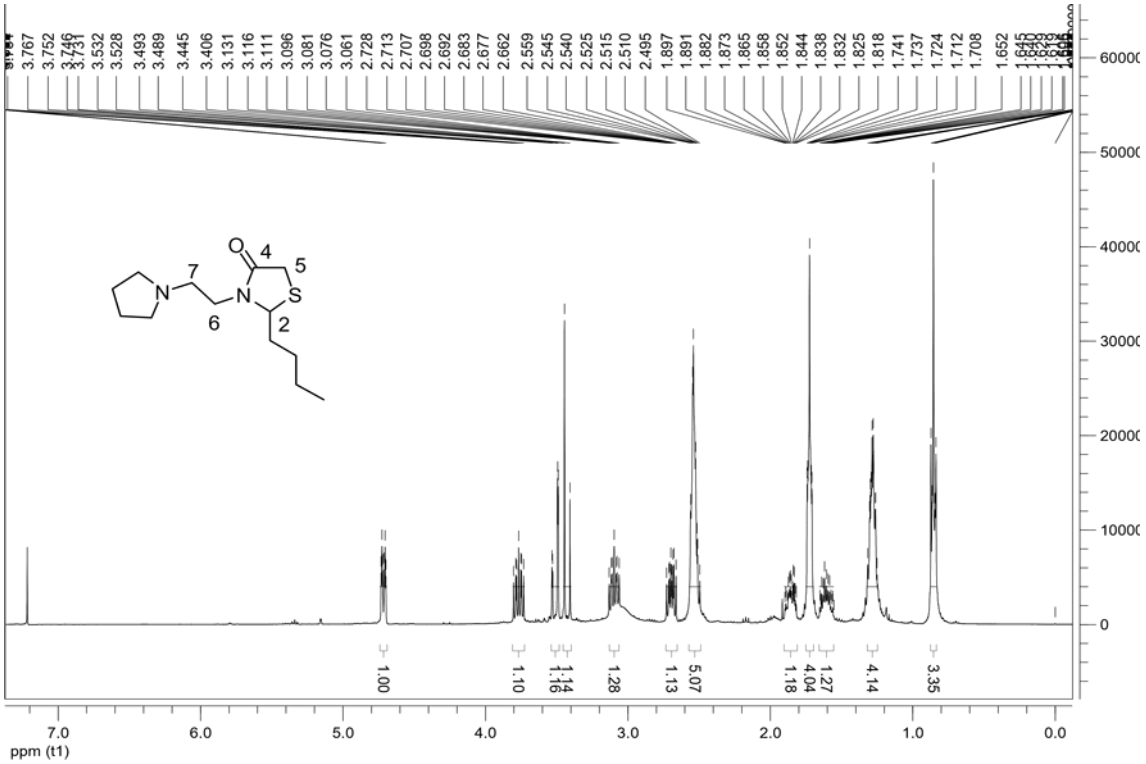

Figure S15: ^{1}H NMR spectrum of compound 2-butyl-3-(2-(pyrrolidin-1-yl)ethyl)thiazolidin-4-one **4Bb**.

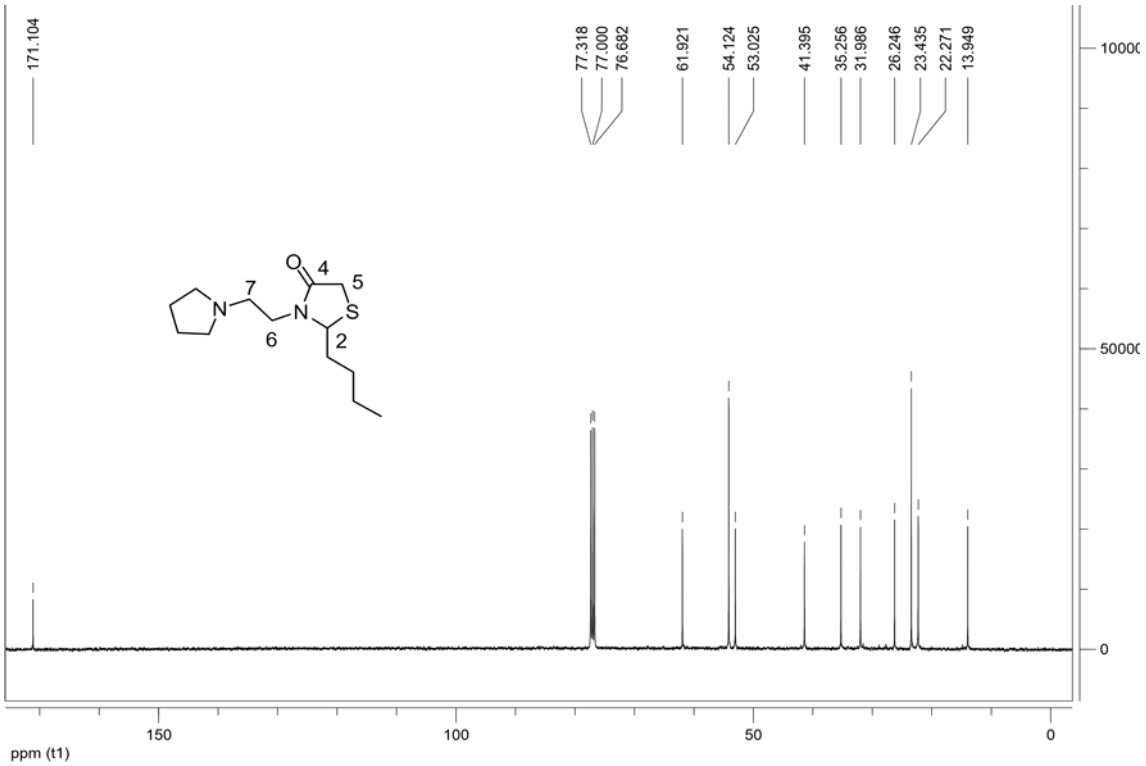

Figure S16: ^{13}C NMR spectrum of compound 2-butyl-3-(2-(pyrrolidin-1-yl)ethyl)thiazolidin-4-one **4Bb**.

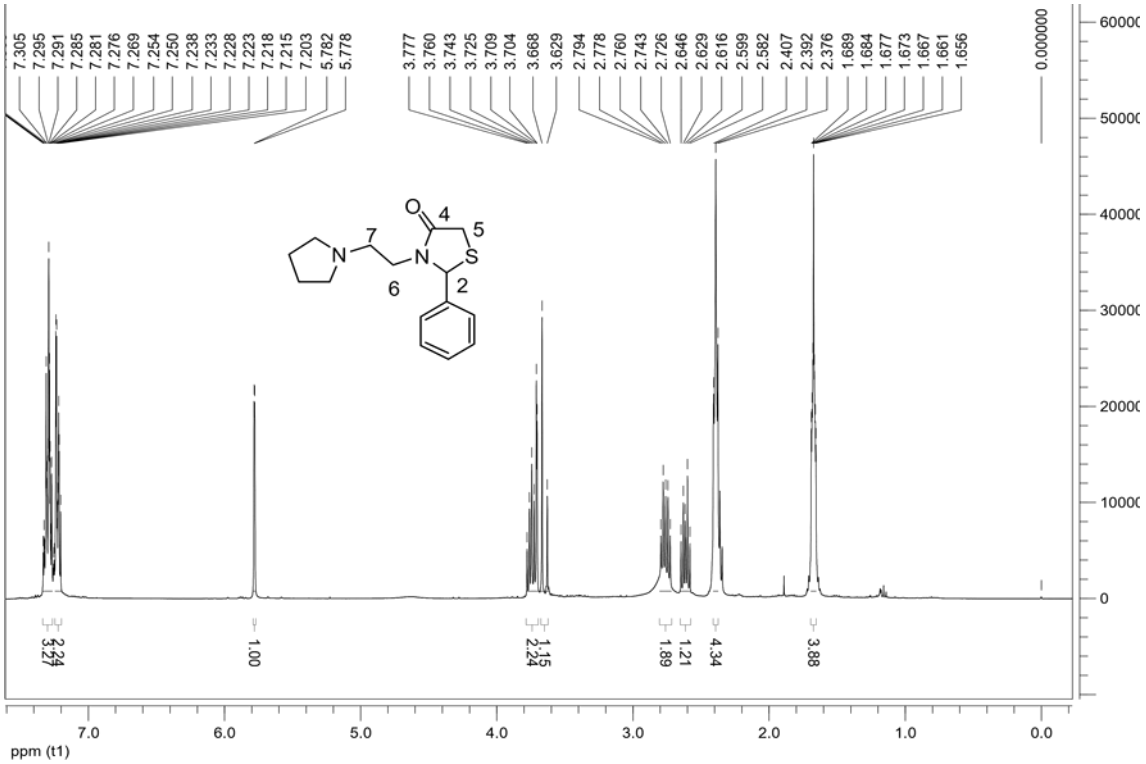

Figure S17: ^{1}H NMR spectrum of compound 2-phenyl-3-(2-(pyrrolidin-1-yl)ethyl)thiazolidin-4-one **4Bc**.

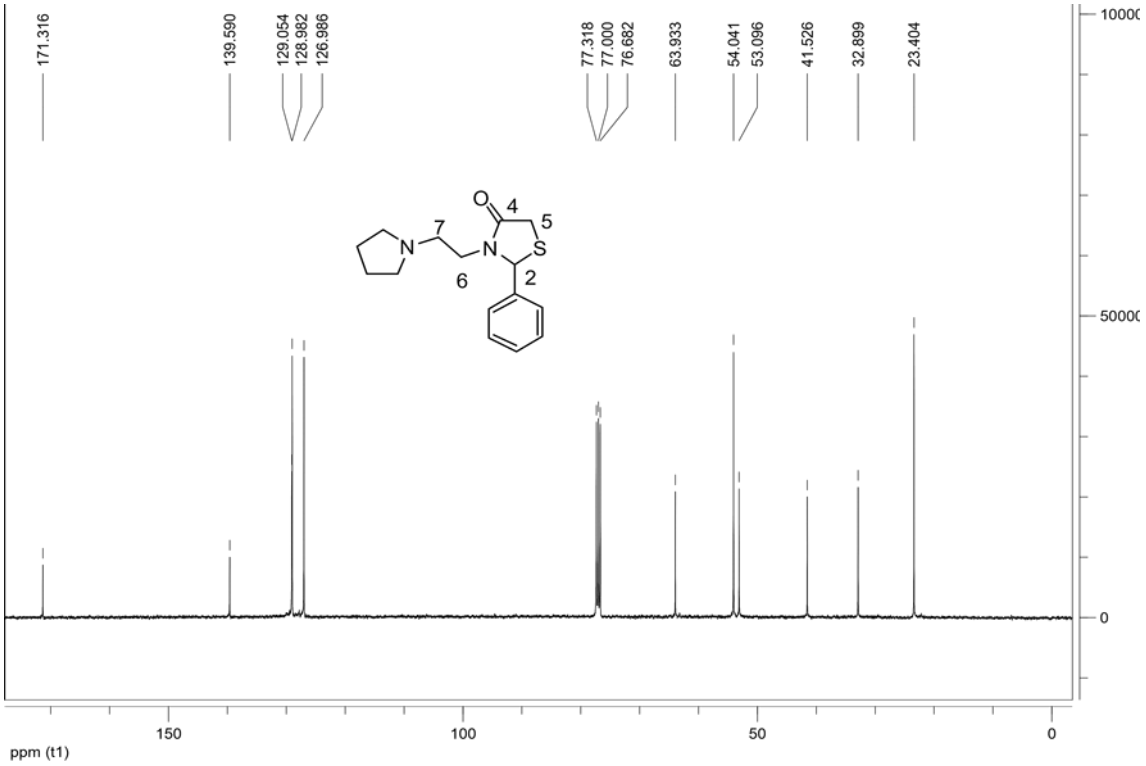

Figure S18: ^{13}C NMR spectrum of compound 2-phenyl-3-(2-(pyrrolidin-1-yl)ethyl)thiazolidin-4-one **4Bc**.

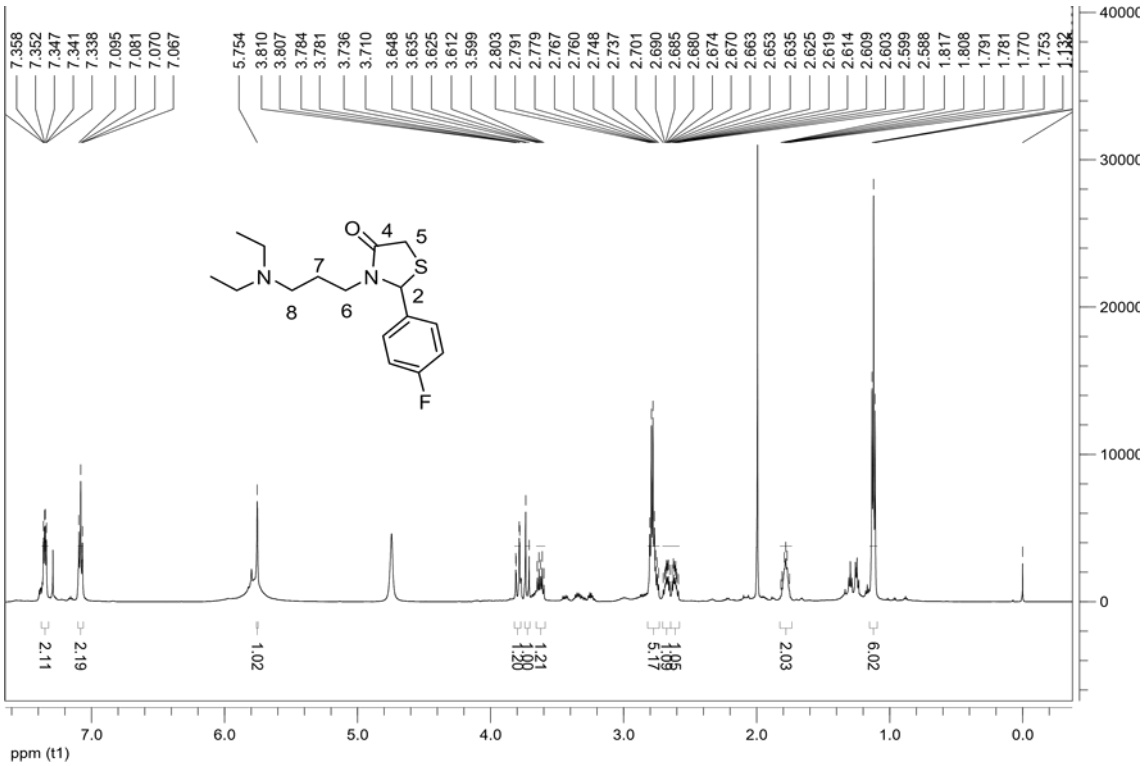

Figure S19: ^{1}H NMR spectrum of compound 3-(3-(diethylamino)propyl)-2-(4-fluorophenyl)thiazolidin-4-one **4Ca**.

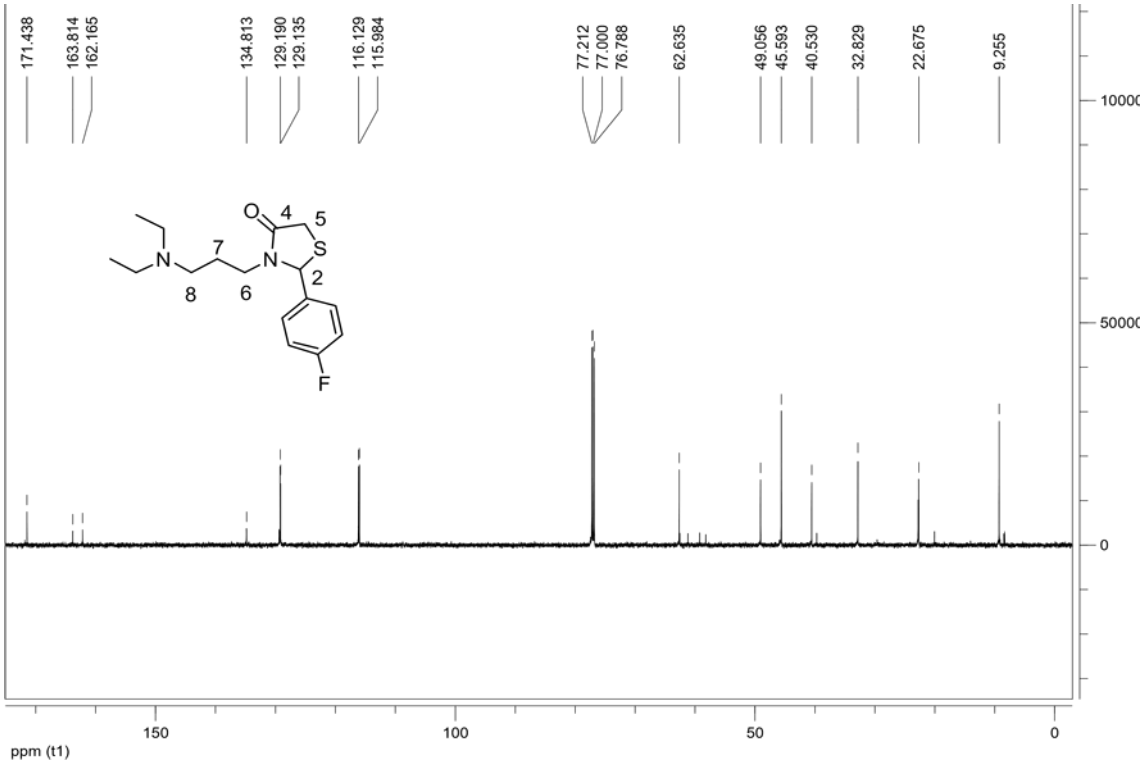

Figure S20: ^{13}C NMR spectrum of compound 3-(3-(diethylamino)propyl)-2-(4-fluorophenyl)thiazolidin-4-one **4Ca**.

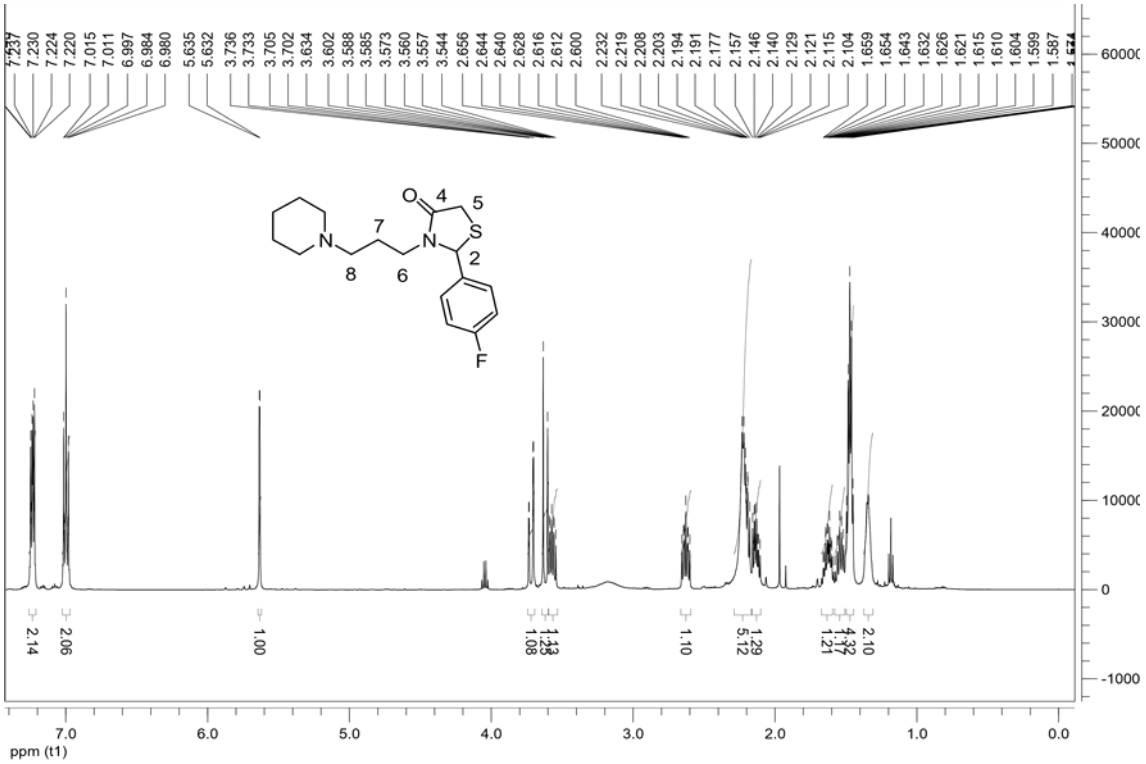

Figure S21: ^{1}H NMR spectrum of compound 2-(4-fluorophenyl)-3-(3-(piperidin-1-yl)propyl)thiazolidin-4-one **4Da**.

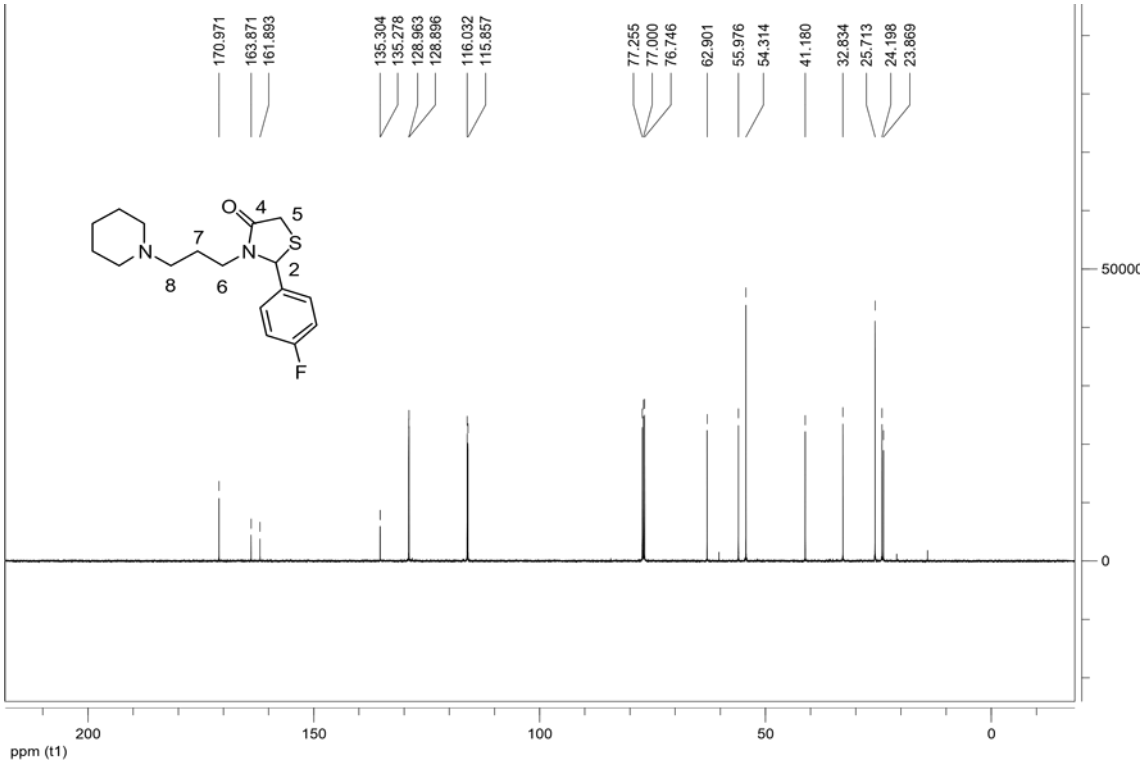

Figure S22: ^{13}C NMR spectrum of compound 2-(4-fluorophenyl)-3-(3-(piperidin-1-yl)propyl)thiazolidin-4-one **4Da**.

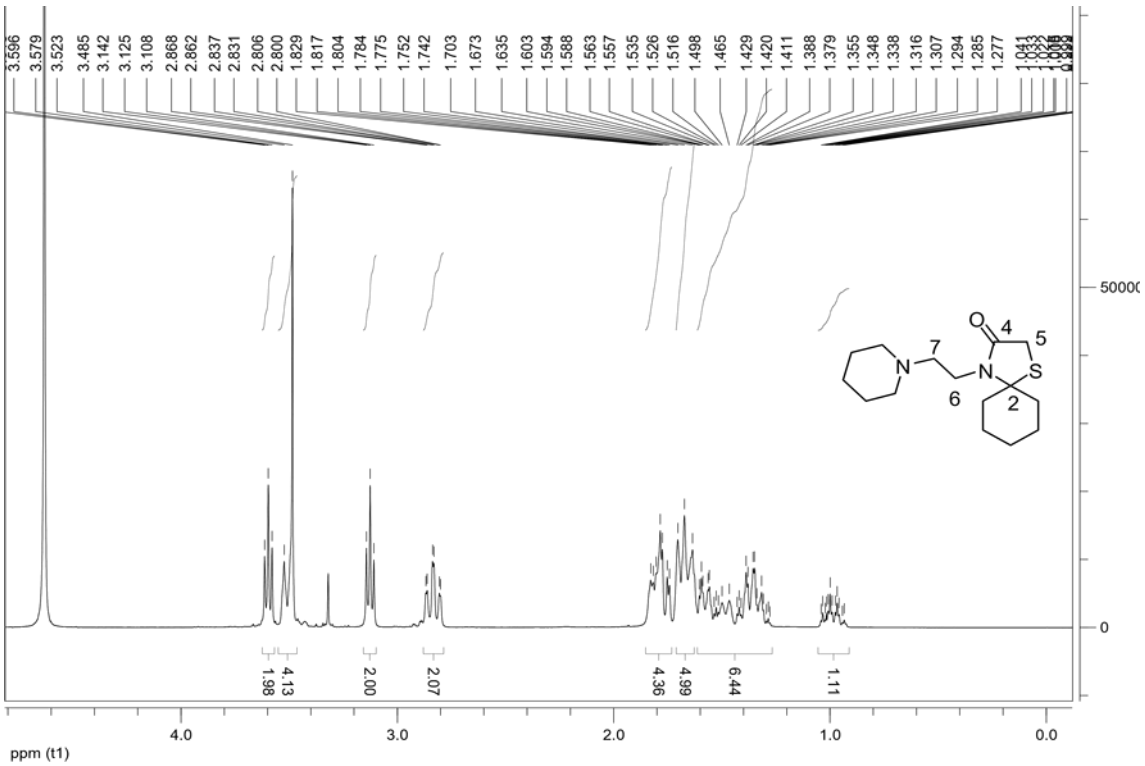

Figure S23: [1]H NMR spectrum of compound 4-(2-(piperidin-1-yl)ethyl)-1-thia-4-azaspiro[4.5]decan-3-one chlorhydrate **5Ad**.

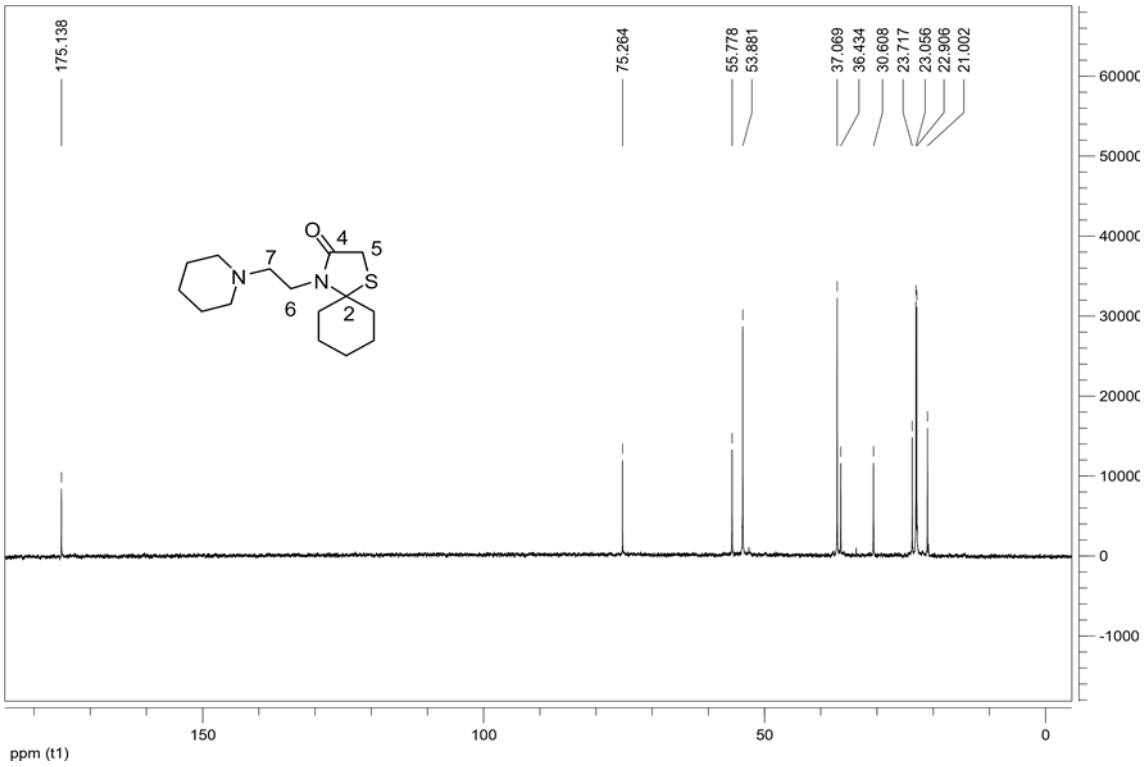

Figure S24: [13]C NMR spectrum of compound 4-(2-(piperidin-1-yl)ethyl)-1-thia-4-azaspiro[4.5]decan-3-one chlorhydrate **5Ad**.

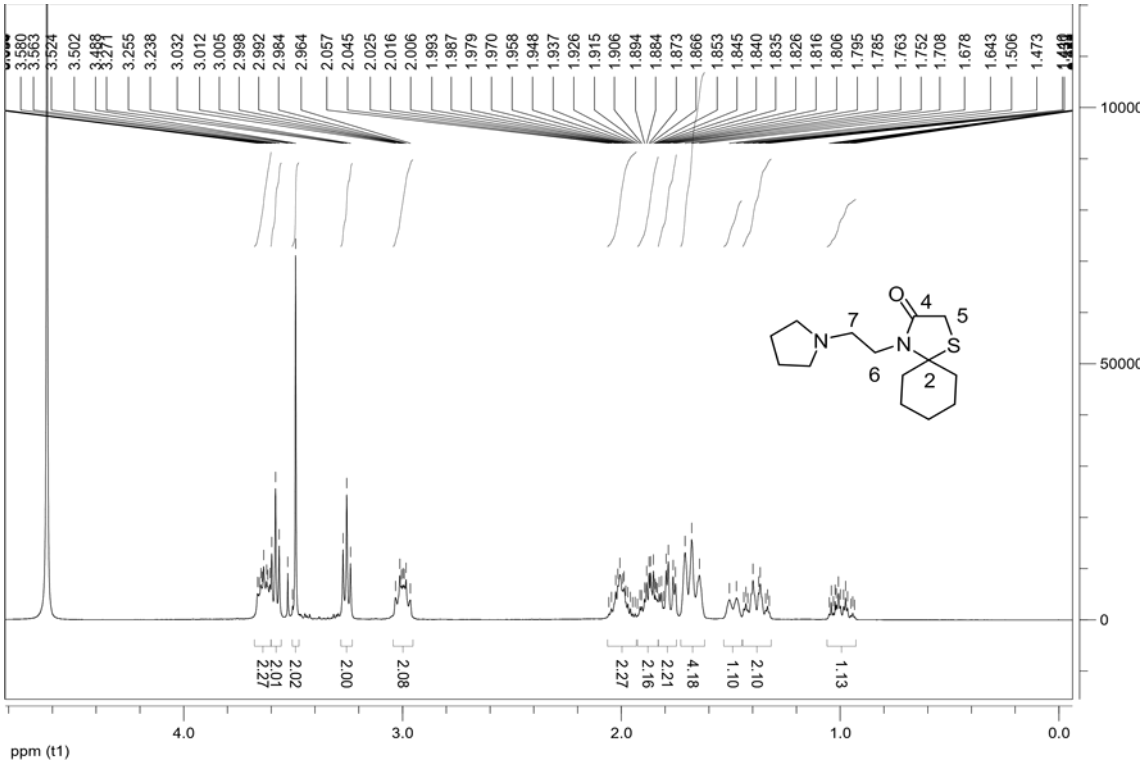

Figure S25: ^{1}H NMR spectrum of compound 4-(2-(pyrrolidin-1-yl)ethyl)-1-thia-4-azaspiro[4.5]decan-3-one chlorhydrate **5Bd**.

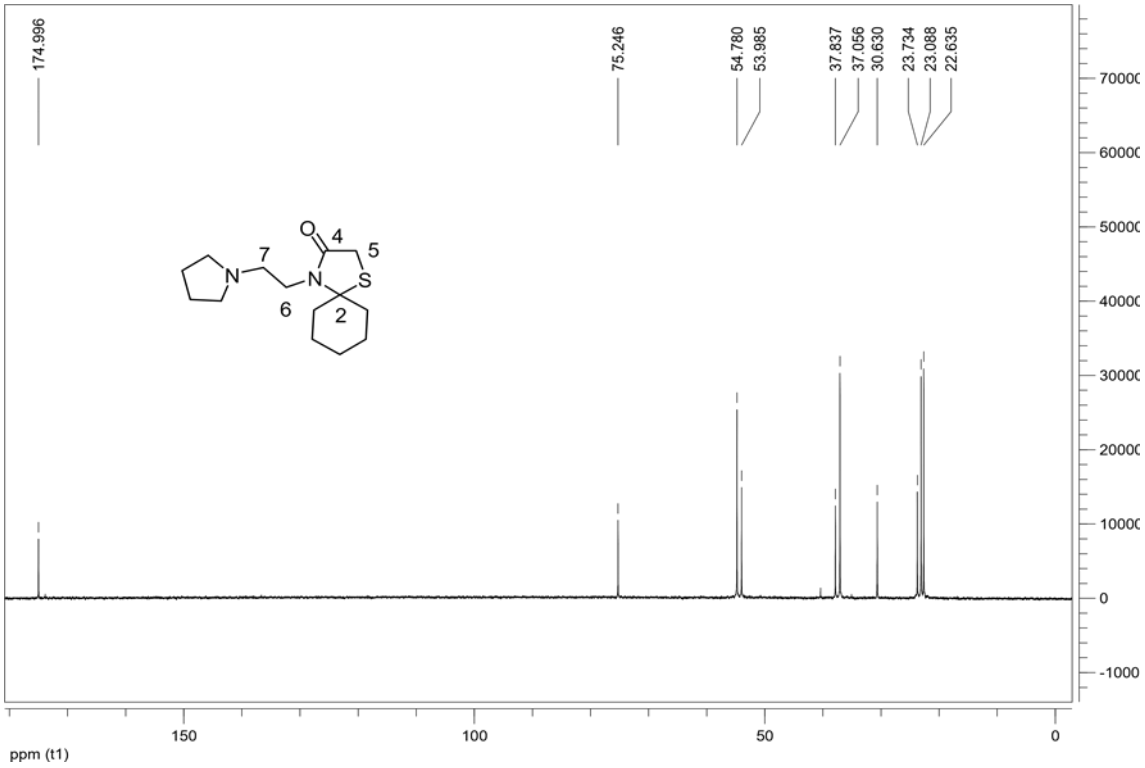

Figure S26: ^{13}C NMR spectrum of compound 4-(2-(pyrrolidin-1-yl)ethyl)-1-thia-4-azaspiro[4.5]decan-3-one chlorhydrate **5Bd**.

5 CONLUSÕES

O potencial antinociceptivo de 2,3-substituido-1,3-tiazolidin-4-onas foi avaliado de modo a evidenciar que estes compostos apresentam propriedades antinociceptivas centrais. Dos quatorze compostos testados nove (**5Aa**, **5Ab**, **5Ac**, **5Ad**, **5Ba**, **5Bb**, **5Bd**, **5Ea**, **5Fa)** demonstraram aumento significativo do tempo de latência no teste da placa quente em comparação a solução salina na avaliação de 30 minutos. Além disso, na comparação com dipirona sódica (500mg/Kg) os derivados **5Ab**, **5Ac** e **5Ad** apresentaram resultados semelhantes, não havendo diferença significativa no tempo de reação na avaliação de 30 minutos. O composto 2-(4-fluorofenil)-3-(piridin-2-ilmetil)-tiazolidin-4-ona (**5Ea**)foi o único que manteve o efeito antinociceptivo na avaliação de 30, 60 e 90 minutos.

Em relação estrutura-atividade, os maiores tempos de latência foram obtidos com a 3-(2-piperidin-1-il) etil)-tiazolidin-4-ona (**5Ab**, **5Ac** e **5Ad**), embora outras aminas também mostraram resultados promissores. O grupo 4-flourfenil (**a**), na posição 2, demonstrou ser um substituinte adequado para indução da nocicepção uma vez que quatro (**5Aa**, **5Ba**, **5Ea** e **5Fa**) dos seis compostos testados obtiveram aumento significativo do tempo de reação na avaliação de 30 minutos. No entanto, os substituintes 2-butil (**b**), 2-fenil (**c**) e 2-ciclo-hexano (**d**) promoveram um maior aumento no tempo de latência do que 4-fluorofenil (a) quando a amina era **A**. Contudo, a substituição de 4-nitrofenil (**e**), ou 4-metoxifenil (**f**) não demonstrou ser favorável para a atividade antinociceptiva destes compostos.

Contudo, são necessários estudos para avaliar a atividade antinociceptiva de formas isoméricas incluindo COX-2. Destaca-se ainda que um novo estudo está sendo realizado para avaliar a atividade analgésica de análogos dos compostos **5Ea** e **5Fa**, bem como a avaliação da atividade anti-inflamatória e antipirética de todos os compostos com resultados significativos. Por fim, a formação do sal cloridrato (**5**) se mostrou adequada para solubilização desses compostos em água já que as bases livres são insolúveis em água.

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[3] G.R. Mettam, L.B. Adams, How to prepare an electronic version of your article, in: B.S. Jones, R.Z. Smith (Eds.), Introduction to the Electronic Age, E-Publishing Inc., New York, 2009, pp. 281–304.
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[4] Cancer Research UK, Cancer statistics reports for the UK. http://www.cancerresearchuk.org/aboutcancer/statistics/cancerstatsreport/, 2003 (accessed 13.03.03).

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