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Inhibition of Mast Cell Leukotriene Release by Thiourea Derivatives

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Abstract—Mast cell derived leukotrienes (LT's) play a vital role in pathophysiology of allergy and asthma. We synthesized various analogues of indolyl, naphthyl and phenylethyl substituted halopyridyl, thiazolyl and benzothiazolyl thioureas and examined their in vitro effects on the high affinity IgE receptor/FcERI-mediated mast cell leukotriene release. Of the **22** naphthylethyl thiourea compounds tested, there were seven active compounds and *N*-[1-(1-naphthyl)ethyl]-*N*′-[2-(ethyl-4-acetylthiazolyl)]thiourea (**17** and **16**) ($IC_{50}=0.002$ μM) and *N*-[1-(1*R*)-naphthylethyl]-*N*′-[2-(5-methylpyridyl)]thiourea (**5**) ($IC_{50}=0.005$ μM) were identified as the lead compounds. Among the 11 indolylethyl thiourea compounds tested, there were seven active compounds and the halopyridyl compounds *N*-[2-(3-indolylethyl)]-*N*′-[2-(5-chloropyridyl)]thiourea and *N*-[2-(3-indolylethyl)]-*N*′-[2-(5-bromopyridyl)]thiourea were the most active agents and inhibited the LTC_4 release with low micromolar IC_{50} values of 4.9 μM and 6.1 μM, respectively. The hydroxylphenyl substituted compounds *N*-[2-(4-hydroxyphenyl)ethyl]-*N*′-[2-(5-chloropyridyl)]thiourea ($IC_{50}=12.6$ μM), *N*-[2-(4-hydroxyphenyl)ethyl]-*N*′-[2-(5-bromopyridyl)]thiourea ($IC_{50}=16.8$ μM) and *N*-[2-(4-hydroxyphenyl)ethyl]-*N*′-[2-(pyridyl)]thiourea ($IC_{50}=8.5$ μM) were the most active pyridyl thiourea agents. Notably, the introduction of electron withdrawing or donating groups had a marked impact on the biological activity of these thiourea derivatives and the Hammett sigma values of their substituents were identified as predictors of their potency. In contrast, experimentally determined partition coefficient values did not correlate with the biological activity of the thiourea compounds which demonstrates that their liphophilicity is not an important factor controlling their mast cell inhibitory effects.

Mast cells participate in the pathophysiology of allergy and asthma through the release of chemical mediators, including the pro-inflammatory leukotrienes (LTs) after crosslinking of their high affinity surface IgE receptors/FcεRI.[1–4] Repeated stimulation of mast cells in patients with allergic asthma may cause sustained synthesis and release of LTs contributing to the significant and persistent bronchoconstriction and inflammatory airway response during episodes of exacerbation. In recent years, several strategies aimed at inhibiting the synthesis and release of leukotrienes (e.g., use of 5-LO inhibitors) or blocking their action at the receptor level (e.g., use of specific LTD_4 antagonists) have been explored as treatment modalities for asthma.[5–8] The purpose of the present study was to examine members of our thiourea compound library[9–12] for mast cell inhibitory activity in an attempt to identify thiourea compounds capable of inhibiting FcεRI-mediated LTC_4 release from mast cells.

Naphthyl-, indolyl- and phenyl-substituted halo pyridyl, thiazolyl and benzothiazolyl thiourea compounds were synthesized according to Scheme 1.[9] The chiral thiourea derivatives were synthesized starting from their respective chiral amines and their chirality was confirmed using optical rotation values. The structures of the compounds were altered by changing the substituents on the pyridyl, thiazolyl and benzothiazolyl rings. Halo and methyl substitutions were chosen to determine the effects of electron withdrawing and donating groups, respectively, on the mast cell inhibitrory activity.

Table 1 shows four different classes of thiourea compounds that were tested for biological activity. The total number of active compounds and the total number of

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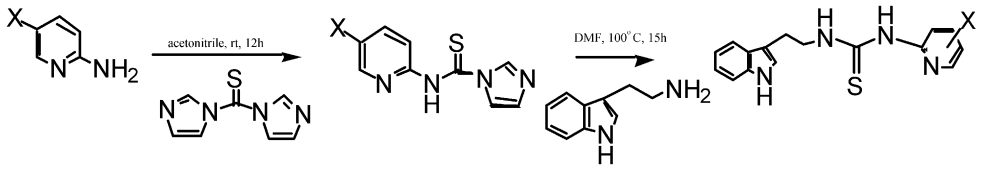


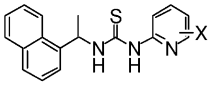
Scheme 1.

Table 1.

Thioureas	Compounds tested	Total number of active compounds	Potent compounds <0.005 μM activity
Class 1	11	2	1
Class 2	11	5	2
Class 3	11	7	0
Class 4	29	5	0

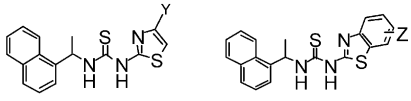
Class 1: chiral naphthyl ethyl pyridyl thioureas; Class 2: chiral naphthyl ethyl thiazolyl, benzothiazolyl thioureas; Class 3: indolyl ethyl pyridyl, thiazolyl and benzothiazolyl thioureas; Class 4: phenylethyl pyridyl, thiazolyl and benzothiazolyl thioureas.

Table 2. (A) Structure–activity of naphthyl pyridyl thioureas



Compd	Isomer	X	Activity in μM
1	*R*	5-Cl	>100
2	*S*	5-Br	>100
3	*S*	5-Br	>100
4	*R*	5-Cl	>100
5	*R*	5-Me	0.005
6	*S*	5-Me	>100
7	*S*	6-Me	1.6
8	*S*	4,6-diMe	>100
9	*R*	4,6-diMe	>100
10	*S*	H	>100
11	*R*	H	>100
WHI-P97	—	—	6.8[a]

(B) Structure–activity of naphthyl pyridyl thioureas



Compd	Isomer	Y	Z	Activity in μM
12	*S*	H	NA	>100
13	*R*	H	NA	>100
14	*S*	Me	NA	83.2
15	*R*	Me	NA	25.9
16	*S*	CH_2COOEt	NA	0.002
17	*R*	CH_2COOEt	NA	0.002
18	*R*	NA	H	18.9
19	*S*	NA	H	>100
20	*S*	NA	4-Me	>100
21	*R*	NA	4-Me	>100
22	*R*	NA	6-OMe	>100

potent compounds are shown in each class. Table 2(A) shows the biological activity observed for chiral naphthyl thioureas. Except for the methyl substituted compounds **5** ($IC_{50}=0.005$ μM) and **7** ($IC_{50}=1.6$ μM), none of these compounds was active ($IC_{50}>100$ μM). Compounds **5** and **7** were more potent than the mast cell inhibitory control dimethoxyquinazoline compound WHI-P97.[13] The most potent compound (**5**) in this series is a eutomer while its enantiomer (**6**) is a distomer. Table 2(B) shows the biological activity of chiral naphthyl thioureas when the pyridyl ring is replaced with a thiazolyl or benzothiazolyl moiety. The thiazolyl substituted compounds with methyl groups inhibited LTC_4 release from IgE/antigen-stimulated mast cells at micromolar concentrations. Notably, the ester analogues **16** and **17** exhibited potent activity with low nanomolar IC_{50} values in the nanomolar range.'*R*' and '*S*' isomers of these thiazolyl-substituted compounds were equally potent implying that stereochemistry does not significantly affect their biological activity. On the other hand, compounds **15** and **18** were found to be eutomers and compounds **14** and **19** were distomers. By comparison, benzothiazolyl-substituted compounds showed only marginal activity. Introduction of methyl or methoxy substituents on the benzothiazolyl moiety eliminated the biological activity of the parent compound. Of the 22 naphthylethyl thiourea compounds tested, *N*-[1-(1-naphthylethyl]-*N*′-[2-(ethyl-4-acetylthiazolyl)]thiourea (**16** and **17**) ($IC_{50}=0.002$ μM) and *N*-[1-(1*R*)-naphthylethyl]-*N*′-[2-(5-methylpyridyl)]thiourea (**5**) ($IC_{50}=0.005$ μM) were identified as the lead compounds. Active inhibitors of LTC_4 release were also identified among indolylethyl thiourea compounds.

Table 1 class 3 compounds show the biological activity observed for indolyl ethyl-substituted thioureas. In the pyridyl substituted compounds, halo substitution produced active compounds. The most active compound in the series was the chloropyridyl substituted thiourea compound. Thiazolyl-substituted compounds were also active albeit with less potency. Interestingly, the ester analogue of thiazolyl substituted compound showed higher potency. Benzothiazolyl compounds in this series were inactive.

Table 1 class 4 compounds show the biological activity obtained for 29 differently substituted phenylethyl thioureas. Only the hydroxy substituted compounds exhibited mast cell inhibitory activity. Substituents such as methyl, fluoro, bromo and chloro yielded inactive compounds, irrespective of the position of substituent in the phenyl ring. We also examined the activity of thiazolyl and benzothiazolyl substituted phenylethyl thiourea compounds and found that none of the compounds in the series showed potent biological activity. Substitutions in the thiazolyl ring or the benzothiazolyl ring did not improve their potency.

We also investigated the role played by various electron donating and electron withdrawing groups on

leukotriene C4 release. A significant correlation was found for the indolyl ethyl substituted compounds. The regression between Hammett and the IC_{50} values showed that introducing electron withdrawing groups such as Br and Cl resulted in more potent compounds that showed a statistically significant relationship between σ and activity ($R^2 = 67\%$, slope $= -1.55 \pm 0.3$, t-ratio $= -5.24$, df $= 13$, $p = 0.0003$).

In summary, among the three classes of thiourea compounds studied for mast cell inhibitory activity, naphthyl substituted thiazolyl thioureas exhibited the most potent activity followed by indolyl ethyl and phenylethyl thiourea compounds. The mast cell inhibitory activity shown by both '*R*' and '*S*' isomers of naphthyl thiourea compounds demonstrate that a few derivatives were found to be eutomers and distomers. In the case of indolyl ethyl substituted thiourea series, the halopyridyl moiety was found to be more beneficial than both thiazolyl and benzothiazolyl units in the structure. Halo substitutions on the pyridyl ring showed improved potency. In the case of phenyl ethyl substituted thioureas, the hydroxy substituent on the phenyl ring showed improved potency compared to other substituents. In addition we found that pyridyl ring in the structure was an essential feature of these phenyl ethyl thiourea compounds. Substituted halopyridyl, indolyl and naphthyl thiourea compounds represent a new chemical class of anti-allergic agents inhibiting IgE/FcRI receptor mediated mast cell LTC_4 release. The lead compounds *N*-[1-(1-naphthylethyl]-*N*′-[2-(ethyl-4-acetylthiazolyl)]thiourea and *N*-[1-(1*R*)-naphthylethyl]-*N*′-[2-(5-methylpyridyl)]thiourea are nanomolar inhibitors of mast cell LTC_4 release.

Statistical Analysis

The IC_{50} values were correlated with Hamett using a linear regression model (JMP software, SAS institute Inc, Cary, NC). Slope parameters were estimated and the t-ratios compared to zero slope were calculated to assess the statistical significance. p values of less than 0.05 were deemed significant.

Stimulation of Mast Cells

RBL-2H3 rat mast cells were sensitized with monoclonal anti DNP IgE antibody (0.24 mg/mL) for 1 h at 37° C in a 48-well tissue culture plate. Unbound IgE was removed by washing the cells with PIPES-buffered saline. After washing, PIPES-buffered saline containing 1 mM calcium chloride was added to the monolayers of the RBL-2H3 cells. The cells were challenged with 20 ng/mL DNP-BSA for 30 min at 37 °C. The plate was centrifuged at 200*g* for 10 min at 4 °C, Supernatants were removed and saved. To study the effect of test drugs, RBL-2H3 rat mast cells were incubated with the drugs at the indicated concentrations or vehicle for 30 min prior to antigen challenge.

Stimulation of Mast Cells and Mediator Assays

RBL-2H3 cells were sensitized with a monoclonal anti-dinitrophenyl (DNP) IgE antibody (0.24 mg/mL) for 1 h at 37 °C in a 48-well tissue culture plate. Unbound IgE was removed by washing the cells with phosphate buffered saline, pH 7.4. After washing the BMMC were re-suspended in RPMI-hepes buffer whereas PIPES-buffered saline containing 1 mM calcium chloride was added to the monolayers of the RBL-2H3 cells. In order to study the biologic effects of the test compounds, sensitized mast cells were further incubated with the test compounds at the indicated concentrations (or vehicle alone) for 1 h. The cells were then challenged with 20 ng/mL DNP-BSA for 30 min at 37 °C. The plates were centrifuged at 200*g* for 10 min at 4 °C. Supernatants were removed and saved. LTC_4 levels were also determined in cell free supernatants.[13,14]

Physicochemical properties of selected thiourea compounds.

***N*-[1-(1*R*)-(1-Naphthylethyl)]-*N*-[2-(5-methylpyridyl)]-thiourea (5).** Mp 186–187 °C; ^{1}H NMR (DMSO-d_6) δ 12.22 (d, 1H, $J = 8.1$ Hz), 10.59 (s, 1H), 8.19 (d, 1H, $J = 8.1$ Hz), 7.92 (d, 2H, $J = 8.4$ Hz), 7.83 (d, 1H, $J = 7.8$ Hz), 7.56–7.46 (m, 5H), 7.06 (d, 1H, $J = 8.4$ Hz), 6.32 (t, 1H), 2.11 (s, 3H), 1.65 (d, 3H, $J = 6.9$ Hz); ^{13}C NMR (DMSO-d_6) δ 178.5, 152.0, 144.9, 139.9, 139.1, 133.6, 130.6, 128.9, 127.9, 127.0, 126.6, 125.8*, 123.4, 122.9, 112.4, 50.5, 21.6, 17.4; IR 3440, 3232, 3027, 2969, 1600, 1562, 1492, 1195, 798 cm^{-1}; UV (MeOH) 203, 222, 250, 272, 294 nm; MALDI-TOF m/z 322.8 ($C_{19}H_{19}N_3S_2 + 2H^+$). [α]−31.0; 5 HPLC R_t: 14.01 min;% purity 99.0; elemental analysis: calcd: C, 70.99; H, 5.96; N, 13.07. Found: C, 71.04; H, 6.04; N, 13.17.

***N*-[1-(1*S*)-(1-Naphthylethyl)]-*N*′-[2-(ethyl-4-acetylthiazolyl)]thiourea (16).** Mp 107–109 °C; ^{1}H NMR (DMSO-d_6) δ 11.54 (s, 1H), 10.10 (s, 1H), 8.15 (t, 1H), 7.96 (t, 1H), 7.86 (t, 1H), 7.61–7.50 (m, 4H), 6.89 (s, 1H), 6.21 (s, 1H), 4.05–4.00 (m, 2H), 3.64 (s, 2H), 1.62 (d, 3H, $J = 3.9$ Hz), 1.16–1.09 (m, 3H); ^{13}C NMR (DMSO-d_6) δ 177.2, 170.3, 161.5, 143.9, 139.3, 134.1, 131.0, 129.4, 128.4, 127.2, 126.5, 126.2, 123.7, 123.3, 109.9, 61.1, 50.5, 31.2, 21.9, 14.9; IR 3444, 3185, 3047, 2927, 1577, 1554, 1519, 1230 cm^{-1}; MALDI-TOF m/z 401.0 ($C_{20}H_{21}N_3O_2S_2 + 2H^+$); [α]: +22.8; HPLC R_t: 6.91 min;% purity 100.0, elemental analysis: calcd: C, 60.13; H, 5.30; N, 10.52. Found: C, 60.06; H, 5.30; N, 10.50.

***N*-[1-(1*R*)-(1-Naphthylethyl)]-*N*′-[2-(ethyl-4-acetylthiazolyl)]thiourea (17).** Mp 105–107 °C; ^{1}H NMR (DMSO-d_6) δ 11.54 (s, 1H), 10.09 (s, 1H), 8.14 (d, 1H, $J = 7.8$ Hz), 7.95 (d, 1H, $J = 7.8$ Hz), 7.86 (t, 1H), 7.62–7.50 (m, 4H), 6.89 (s, 1H), 6.20 (t, 1H), 4.02 (q, 2H), 3.63 (s, 2H), 1.62 (d, 3H, $J = 5.4$ Hz), 1.12 (t, 3H); ^{13}C NMR (DMSO-d_6) δ 176.5, 169.7, 160.8, 143.3, 138.7, 133.5, 130.4, 128.8, 127.8, 126.5, 125.9, 125.6, 123.1, 122.7, 109.4, 60.5, 49.9, 36.6, 21.3, 14.3; IR 3463, 3166, 2981, 1743, 1573, 1535, 1508, 1380, 1211, 1164, 794 cm^{-1}; MALDI-TOF m/z 401.2 ($C_{20}H_{21}N_3O_2S_2 + 2H^+$); [α]−25.5; HPLC R_t: 9.89

min;% purity 99.9, elemental analysis: calcd: C, 60.13; H, 5.30; N, 10.52. Found: C, 60.40; H, 5.36; N, 10.53.

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