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Synthesis and rational design of anti-inflammatory compounds: *N*-phenyl-cyclohexenyl sulfonamide derivatives

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The synthesis of (±)-ethyl 6-[*N*-(2-chloro-4-fluorophenyl)sulfamoyl]cyclohex-1-ene-1-carboxylate (5n) has been reproduced from a method previously described and served as the background for the preparation of a nitro derivative, potentially useful as an anti-inflammatory agent. Furthermore, a structure-based QSAR analysis of a series of *N*-arylsulfamoyl congeners derived a highly predictive model for the activities of novel small-molecule inhibitors of NO and cytokine production, whose preparation may be successfully achieved according to a similar procedure as above. Copyright © 2009 John Wiley & Sons, Ltd.

Keywords: anti-inflammatory compounds; MIA-QSAR; *N*-arylsulfamoyl derivatives

INTRODUCTION

Sepsis is a systemic inflammatory response syndrome induced by bacterial infection, and is assumed to be one of the main causes of death in intensive care units, with mortality rates between 30 and 70%.[1–3] It is caused by bacterial components which induce host immune cells to activate and stimulate the production of various kinds of mediators, such as nitric oxide (NO) and cytokines.[4–7] Thus, regulation of proinflammatory mediator production would be a critical process to combat various inflammatory diseases.

Ethyl (6*R*)-6-[*N*-(2-chloro-4-fluorophenyl)sulfamoyl]cyclohex-1-ene-1-carboxylate (TAK-242, Fig. 1) has been recently implemented as a small molecule cytokine production inhibitor.[8] It is derived from a series of alkyl 6-(*N*-substituted sulfamoyl)cyclohex-1-ene-1-carboxylates previously reported;[9] a structure-based analysis revealed that a chlorine atom at 2-position in the phenyl ring causes a positive effect on bioactivity, as does a fluorine atom at the 4-position. This suggests that a different electronegative substituent, such as the nitro group, should lead to similar biological activity. In addition, the congeneric series offers the possibility of building a ligand-based QSAR model, which may be useful to derive novel compounds with high anti-inflammatory potency.

Among the structure-based QSAR methods, MIA-QSAR (multivariate image analysis applied to quantitative structure–activity relationships) has provided highly predictive models, comparable to 2D classical and sophisticated 3D methodologies.[10–16] Thus, the goal of this work was to synthesize a novel derivative of the highly potent TAK-242, and also to develop a QSAR model based on the scaffold of this compound, in order to propose new congeners with significantly higher activity.

EXPERIMENTAL

Synthesis and NMR

The synthesis of ethyl (±)-6-[*N*-(2-chloro-4-fluorophenyl)sulfamoyl] cyclohex-1-ene-1-carboxylate (**5n**), the racemic form of TAK-242, was reproduced from the procedure described elsewhere.[8] A similar procedure was performed to obtain the 3-nitro derivative, according to the following description (Scheme 1). ^{1}H and ^{13}C NMR spectra were acquired on a Varian GEMINI 300 spectrometer, operating at 300.07 and 75.45 MHz, respectively. Samples were prepared as solutions of 20 mg of solute in 0.6 ml of DMSO-d_6 solvent, and the spectra were recorded at 300 K.

Ethyl 2-mercaptocyclohex-1-ene-1-carboxylate (**2**)

Hydrogen sulfide (H_2S) gas was bubbled through a solution of ethyl 2-oxocyclohexanecarboxylate (**1**) (20 g, 117 mmol) in

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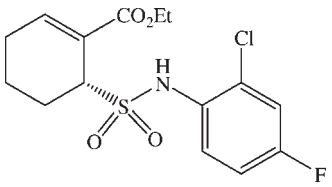


Figure 1. Structure of TAK-242

ethanol (300 ml) at −50 °C for 2 h, and then hydrogen chloride (HCl) gas was bubbled into the reaction mixture at −20 °C for 2 h, followed by the bubbling of H_2S gas at −20 °C for 2 h. After having been allowed to stand for 14 h, the reaction mixture was poured into ice water (200 ml) and worked up [hexane (600 ml); water (5 × 160 ml)]. The residue was purified by distillation (bp 136–139 °C/15–16 mmHg (bp 134–135 °C/15–16 mmHg) to give **2** (14.2 g, 65%) as a colorless oil. ^{1}H NMR ($CDCl_3$), δ (ppm): 1.30 (3H, t, J = 7.1 Hz), 1.55–1.70 (4H, m), 2.17–2.30 (4H, m), 4.20 (2H, q, J = 7.1 Hz), 12.3 (1H, s). ^{13}C NMR ($CDCl_3$), δ (ppm): 14.3 (CH_3), 21.9 (C-6), 22.4 (C-4 and C-5), 29.1 (C-3), 60.1 (CH_2-O), 97.8 (C-1), 172.0 (C-2), 172.8 (C═O).

2-(Ethoxycarbonyl)cyclohex-1-ene-1-sulfonic acid (**3**)

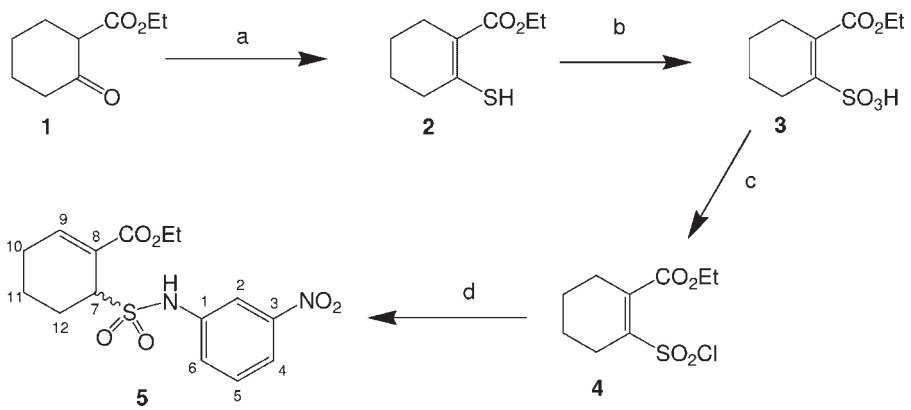
A solution of **2** (7.0 g, 37.6 mmol) in AcOH (12.5 ml) was added dropwise to a stirred solution of sodium borate tetrahydrate (18.6 g, 121 mmol) in AcOH (100 ml) for 2 h at 50–55 °C. The resulting mixture was stirred for 3 h at 50–55 °C and for 9 h at 80–85 °C, and then cooled and evaporated under reduced pressure to dryness. Acetonitrile (165 ml) was added to the residue, and the whole residue was stirred for 1 h followed by filtration to remove the insoluble substances. After the filtrate was evaporated under reduced pressure to dryness, the residue was dissolved in MeCN (125 ml) and then stirred for 2 h. The resulting precipitate was filtered off, and the filtrate was evaporated under reduced pressure to dryness. Diisopropyl ether (330 ml) was added to the residue to obtain **3** (5.6 g, 63%) as a white powder: ^{1}H NMR (DMSO-*d6*), δ (ppm): 1.17 (3H, t, J = 7.1 Hz), 1.50 (4H, br), 2.08–2.09 (2H, m), 2.22–2.24 (2H, m), 3.99 (2H, q, J = 7.1 Hz). ^{13}C NMR ($CDCl_3$), δ (ppm): 13.9 (CH_3), 21.2 (C-4), 21.7 (C-5), 24.3 (C-3), 27.2 (C-6), 59.7 (CH_2-O), 128.2 (C-1), 139.9 (C-2), 170.5 (C═O).

Ethyl 6-[N-(3-nitrophenyl)sulfamoyl]cyclohex-1-ene-1-carboxylate (**5**)

Compound **3** (2.0 g, 8.3 mmol) obtained above was dissolved in thionyl chloride (5.4 ml) and heated under reflux for 14 h, and then the reaction mixture was evaporated under reduced pressure to dryness. The residue was subjected three times to a procedure involving an addition of hexane (7.7 ml) followed by evaporation under reduced pressure to dryness to yield ethyl 2-(chlorosulfonyl)cyclohex-1-ene-1-carboxylate (1.2 g; 4.7 mmol). This residue (1.2 g) was combined with ethyl acetate (4 ml), and the resultant mixture was added to a solution of 3-nitro-aniline (1.0 g, 6.73 mmol), Et_3N (1 ml, 6.73 mmol), and ethyl acetate (10 ml) and then stirred for 18 h. The reaction mixture was diluted with ethyl acetate (9.5 ml), and the entire mixture was then worked up [water (40 ml), brine (20 ml × 3)]. The residue was crystallized from diisopropyl ether (1.5 ml), and the precipitate was washed with diisopropyl ether (2.0 ml) and ethyl acetate (1.6 ml) to yield **5** (120 mg, 4.1%) as colorless crystals: ^{1}H NMR (CD_2Cl_2), δ (ppm): 1.28 (3H, t, J = 7.1 Hz), 1.62–1.85 (2H, m), 1.89–2.40 (4H, m), 4.20 (2H, q, J = 7.1 Hz), 4.32 (1H, d, J = 4.2 Hz), 5.34 (1H, s), 7.38 (1H, t, J = 3.8 Hz), 7.68 (1H, ddd, J = 1.0, 2.1, 8.2 Hz), 8.0 (1H, ddd, J = 1.0, 2.1, 8.2 Hz), 8.18 (1H, t, J = 2.0 Hz). ^{13}C NMR ($CDCl_3$), δ (ppm): 13.6 (CH_3), 20.9 (C-12), 24.7 (C-11), 27.2 (C-10), 59.3 (C-7), 64.4 (CH_2), 112.8 (C-2), 116.4 (C-4), 124.8 (C-6), 129.2 (C-5), 129.6 (C-8), 130.6 (C-9), 140.6 (C-1), 147.3 (C-3), 169.4 (C(═O)).

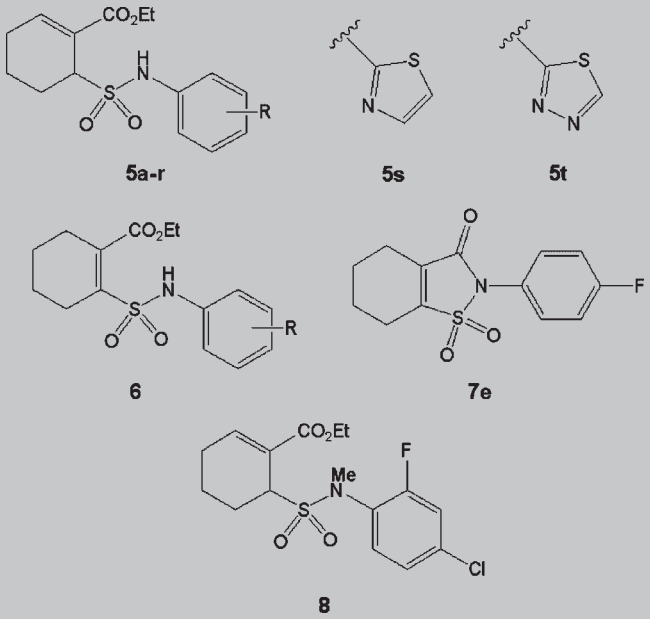
MIA-QSAR modeling

MIA descriptors are binaries, i.e., pixels of 2D images; in QSAR, such images are chemical structures drawn using an appropriate drawing program, where the substituents attached to a scaffold correspond to the explained variance in the data. In this work, the 25 anti-inflammatory compounds (Table 1), a series of *N*-arylsulfamoyl derivatives obtained from the literature,[9] were drawn using the ChemSketch program.[17] Subsequently, the chemical structures were transformed into bitmaps and saved in a 370 × 300 pixels workspace. Since the dataset used is a congeneric series, chemical structures contain a common substructure, which was superimposed for the entire series. This was achieved by taking a pixel in common among the whole series of compounds and fitting it in a given coordinate of the defined workspace. This 2D alignment is rapid and requires only



Scheme 1. Synthesis of ethyl 6-[*N*-(3-nitrophenyl)sulfamoyl]-cyclohex-1-ene-1-carboxylate (**5**). Reagents: (a) H_2S/HCl, EtOH; (b) $NaBO_3.4H_2O$, acetic acid; (c) $SOCl_2$; (d) 3-nitroaniline, Et_3N, AcOEt

Table 1. Compounds used in the MIA-QSAR modeling, and the respective experimental, calibrated, and predicted biological activities (pic_{50})



Compound	R	Exp.	Cal.	LOO CV	L-20%-O CV
5a	2-F, 4-Cl	6.796	6.885	6.935	6.898
5b	H	6.585	6.674	6.729	6.891
5c	2-F	7.125	7.152	7.142	7.195
5d	3-F	6.824	6.757	6.647	6.316
5e	4-F	6.959	7.410	7.621	7.466
5f	2-Cl	7.921	7.540	7.267	7.036
5g	3-Cl	7.180	6.848	6.440	6.194
5h	4-Cl	6.398	6.432	6.658	6.962
5i	2,3-F_2	6.854	7.241	7.395	7.381
5j	2,6-F_2	6.796	6.819	7.116	7.142
5k	2,4-F_2	7.796	7.958	7.930	7.948
5l	2,4,5-F_2	7.523	7.669	7.515	7.373
5m	2,3-Cl_2	7.699	7.291	6.974	7.078
5n	2-Cl, 4-F	8.495	8.262	7.828	7.885
5o	2-Cl, 4-Me	7.387	7.479	7.323	6.939
5p	2-Cl, 4-CN	5.796	5.752	6.809	6.956
5q	2-Et	6.886	6.868	6.881	6.717
5r	2-CO_2Me	5.959	5.851	6.375	6.384
5s	—	5.000	4.834	5.210	5.398
5t	—	5.000	5.105	4.677	4.725
6a	2-F, 4-Cl	5.770	5.624	5.818	5.931
6b	H	5.086	5.490	6.017	5.864
6e	4-F	5.854	5.649	5.619	5.631
7e	—	5.387	5.433	5.266	5.387
8	—	6.638	6.681	6.986	6.973

simple manual precision. The 25 2D images were appropriately read and converted into double arrays by using Matlab.[18] These 25 samples were then grouped to give a $25 \times 370 \times 300$ three-dimensional array, and then unfolded to give an **X**-matrix of $25 \times 111\,000$ dimension. The MIA-QSAR model was built by calibrating the **X**-matrix with the corresponding dependent variables (inhibitory activities on NO production—pIC_{50}, IC_{50} in $mol \cdot L^{-1}$) by using the PLS regression method. Model validation was achieved through cross-validation (LOO and L-20%-O), and the predictive ability was statistically evaluated through the root

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mean square errors of calibration (RMSEC) and cross-validation (RMSECV), as well as by the squared correlation coefficients of the regression line of experimental *versus* fitted (r^2) or predicted (q^2) activity values.

RESULTS AND DISCUSSION

It has been found that a chlorine atom bonded to the 2-position in the phenyl ring of the titled aryl-sulfamoyl compounds plays a key role in enhancing the biological activities of the congeners in Table 1; a fluorine in the 4-position activates the compound and makes ethyl 6-[*N*-(2-chloro-4-fluorophenyl)sulfamoyl]cyclohex-1-ene-1-carboxylate the most active compound of Table 1 (the *R* enantiomer, TAK-242, has been separated and shown to display a higher potency). In addition to the available data for the anti-inflammatory activity of TAK-242, preliminary bioassays with this drug have shown a decrease in the Jun NH_2-terminal kinase (JNK) phosphorylation in the liver and adipose and muscular tissues, indicating that TAK-242 reduces the anti-inflammatory sub-clinic process in obese animals. Treatment for 14 days decreased significantly the phosphorylation in the liver (65%, $p < 0.05$), skeletal muscle (67%, $p < 0.05$) and adipose tissue (35%, $p < 0.05$). Further biological experiments with the 3-nitro derivative and others are currently in progress.

The synthetic route applied to obtain TAK-242 was reproduced, and then used to derive a model example, the 3-nitro derivative, in order to show that the synthesis may be expanded to other analogous systems. 3-Nitro-aniline was chosen as the starting reagent for the synthesis because the nitro substituent is electronegative and reasonably voluminous, such as the chlorine and fluorine of TAK-242, and is supposed to affect the bioactivity similarly. The synthetic sequence of Scheme 1 may be used to carry out syntheses of novel compounds, which are based on the basic structure of Table 1. Such new entities may be appropriately rationalized by combining the substructures, in this case the substituents, of a congeneric series of aryl-sulfamoyl compounds—those of Table 1; their activities can be computationally predicted by using a suitable structure-based QSAR method. MIA-QSAR has been applied elsewhere to give highly predictive models,[10–16] and then it has been used here to model the activities of the compounds of Table 1. Moreover, some prototypes were proposed and their activities predicted by using the PLS regression parameters.

First, the optimum number of latent variables (linear combinations of the original variables—the pixels) to be used in the model was achieved by analyzing the variance in RMSEC and RMSECV when changing the number of PLS components (Fig. 2); the minimum error was found for five latent variables. The calibration step for the entire dataset of Table 1 gave the fitted pIC_{50} values of Table 1, corresponding to a correlation with the experimental data of r^2 0.945 (RMSEC = 0.218) (Fig. 3). The calibration model was validated through leave-one-out and leave-20%-out cross-validations; in this latter validation procedure, samples were randomly selected into five segments. The model was found to be satisfactorily predictive, with q^2_{LOO} of 0.757 (RMSECV = 0.462) and $q^2_{L\text{-}20\%\text{-}O}$ of 0.694 (RMSECV = 0.517), according to the predicted values of Table 1, as illustrated in Fig. 3.

Given the prediction ability of the QSAR model, the PLS regression parameters were used to estimate the bioactivities of novel structures, which are variations of the common scaffold

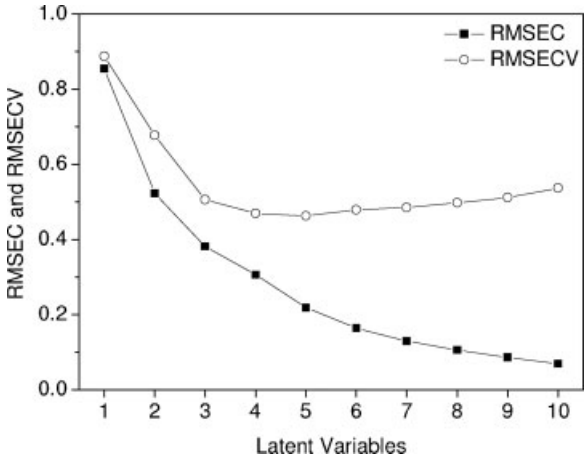

Figure 2. Plot of number of latent variables *versus* RMSEC/RMSECV

and substituents which have the greatest influence on the biological activities. The novel compounds proposed have di- and tri-substituted phenyl rings (substituents = F and Cl, at the 2, 4, and/or 5 ring-position), as shown in Fig. 4. These respective positions for the substituents were calibrated, and thus the predictions should provide reliable (within RMSECV) calculated activities. The predicted pIC_{50} values for the prototype compounds **A**, **B**, and **C** of Fig. 4 were 6.717, 6.847, and 7.099, which are within the range of moderately and highly active compounds of the dataset. This suggests that the proposed new compounds are potentially useful as starting systems for further structure modification.

Overall, the MIA-QSAR model built was found to be highly predictive for the series of aryl-sulfamoyl compounds presented. It may be applied to the prediction of novel congeners, whose synthesis is expected to proceed successfully by using a similar procedure as described in the literature and tested here for a model system (the 3-nitrophenyl derivative). Furthermore, the computational approach demonstrated its potential to drive synthesis and, in combination with experimental procedures, is capable of diminishing time and costs during drug development.

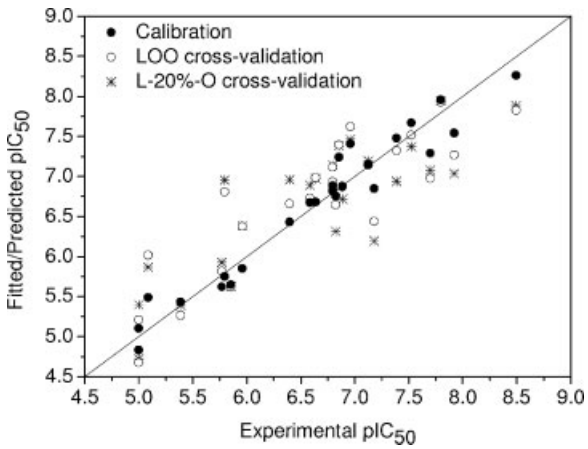

Figure 3. Correlation of experimental *versus* calibrated/predicted pIC_{50}

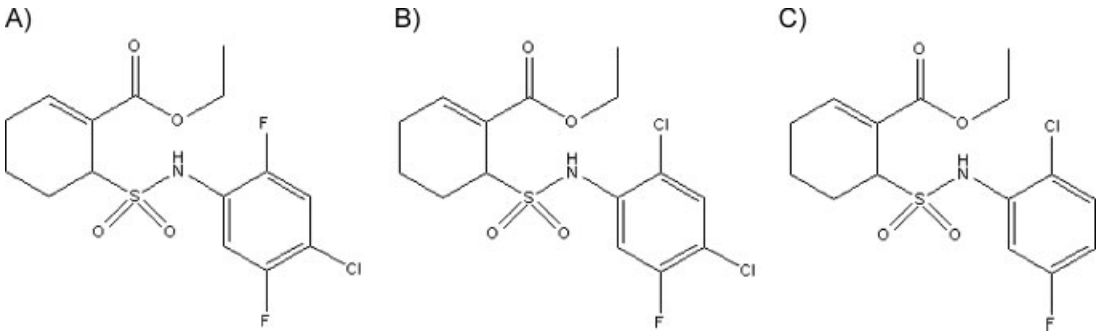

Figure 4. Proposed compounds with inhibitory activities on NO production, as modeled through MIA-QSAR

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