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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Three-dimensional common-feature hypotheses for hypoglycemic flavonyl-2,4-thiazolidinedione derivatives

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Abstract Three-dimensional pharmacophore hypotheses were built from a set of seven antidiabetic agents. Among the ten commonly featured models generated by program Catalyst/HipHop, a hypothesis including four hydrogen-bond acceptors (HBAs) and two hydrophobic aromatics (HpArs) features was considered to be important in evaluating the hypoglycemic activity. The most active 3-ethyl-5-[3′-(4H-4-oxo-1-benzopyran-2-yl)benzylidene]-2,4-thiazolidinedione (**4b**) mapped well onto all the HBAs and HpArs features of the hypothesis.

Keywords Pharmacophore analysis · HipHop · Hypoglycemic agents · 2,4-thiazolidinediones · 2,4-imidazolidinediones · 2-thiohydantoin · Flavone

Introduction

Type 2 diabetes is one of the most common metabolic diseases that lacks fully effective therapy and is characterized by abnormalities of insulin secretion and by insulin resistance of major target tissues (DeFronzo *et al.*, 1992; Yki-Järvinen, 1994). 2,4-Thiazolidinediones (2,4-TZDs) are a new class of antidiabetic agents, differ markedly from other antidiabetic agents in that they are effective in normalizing glucose and lipid metabolism associated with insulin resistance, and therefore are expected to be useful in the treatment of both type 2 diabetes mellitus and obesity (Sohda *et al.*, 1990; Iwamoto *et al.*, 1991; Suter *et al.*, 1992). The prototypical agent, ciglitazone, was shown to normalize hyperglycemia in insulin-resistance models without affecting glycemia in nondiabetic animals (Fujita *et al.*,

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1983). After extensive evaluation of numerous compounds, several other agents were developed, including pioglitazone, englitazone, and troglitazone. All of these compound posses a common 2,4-thiazolidinedione structure, but they differ in side chain that influences their pharmacological actions and potential for adverse effects.

Troglitazone, the first drug launched in this series, was withdrawn recently from the market after a request by the Food and Drug Administration because of its idiosyncratic hepatotoxicity in patients (Food and Drug Administration, 2000). Therefore, there is a greater need to develop a safe and effective insulin sensitizer for type 2 diabetes. By decreasing insulin resistance, thiazolidinediones offer a promising new approach to the treatment of diabetes.

A daunting task for the researcher today is to decipher how structurally diverse molecules can bind at a common receptor site. When considering a receptor of unknown structure, an analysis of the ligand set will be highly dependent on both the choice of active conformation, and the proposed alignment of these molecules with respect to one another. Although the molecules belong to different structural classes of compounds, they may contain a common three-dimensional (3-D) arrangement of features. The Catalyst program HipHop generates a set of common feature pharmacophore models from a set of compounds known to be active at a specific therapeutic area (Clement and Mehl, 2000).

The purpose of this article was to derive feature-based 3-D models from a set of seven active compounds, which had 3′(or 4′)-flavonyl-2,4-thiazolidinedione or its analogues, such as 2,4-imidazolidinedione and 2-thiohydantoin, taking from Bozdağ *et al.*, (2000a, b) responsible for the insulin-releasing activity on INS-1 cells. Among the ten common-featured models generated by the program Catalyst/HipHop (Accerlys Inc., San Diego, Ca, 2004), a hypothesis, including four hydrogen-bond acceptors (HBAs) and two aromatic hydrophobes (HpArs) features, was considered to be essential for hypoglycemic activity.

Hypothesis generation

All computational experiments were conducted on a Silicon Graphics O2, running under the IRIX 6.5 operating system. Hypotheses generation was applied against previously described data sets by using Catalyst/HipHop (version 4.9) from Accerlys Inc., San Diego, CA, 2004. Molecules were edited using the Catalyst 2D/3D visualizer. Catalyst automatically generated conformational models for each compound using the Poling Algorithm (Smellie et al., 1994, 1995a, b). The "best conformer generation" procedure was applied to provide the best conformational coverage for a maximum number of conformers generated defaulted to 250 in a 0–20 kcal/mol range from the global minimum. The conformations generated were used to align common molecular features and generate pharmacophore hypotheses. HipHop used conformations generated to align chemically important functional groups common to the molecules in the study set. A pharmacophoric hypothesis was generated from these aligned structures.

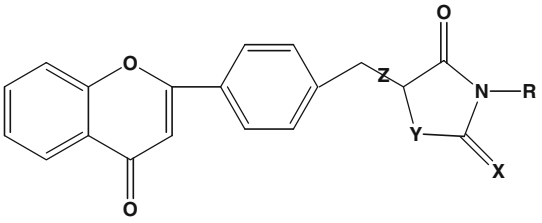
HipHop provides feature-based alignment of a collection of compounds without considering activity. It matches the chemical features of a molecule against drug

candidate molecules. HipHop takes a collection of conformational models of molecules and a selection of chemical features and produces a series of molecular alignments in a variety of standard file formats. HipHop begins by identifying configurations of features common to a set of molecules. A configuration consists of a set of relative locations in 3-D space and associated feature types. A molecule matches the configurations if it possesses conformations and structural features that can be superimposed within a certain tolerance from the corresponding ideal locations. HipHop also maps partial features of molecules in the alignment set. This provision gives the option to use partial mapping during the alignment. Partial mapping allows identification of larger, more diverse, more significant hypotheses and alignment models without the risk of missing compounds that do not have to map to all of the pharmacophore features. Misses, the number of molecules that do not have to map to all features in generated hypotheses, FeatureMisses, the number of maximal molecules that do not have to map to each feature in generated hypotheses, and CompleteMisses, the number of molecules that do not have to map to any feature in a given hypothesis, were set as 3, 2, and 2, respectively.

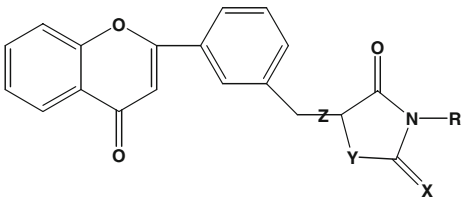
Results and discussion

In vitro insulin secretion effect in INS-1 cells of some novel 3′ (or 4′)-flavonyl-2,4-thiazolidinedione/2,4-imidazolidinedione/2-thiohydantoin 20 compounds were investigated (Table 1) (Bozdağ *et al.*, 2000a, b). Hypotheses were generated to explain the specificity of the hypoglycemic agents. A set of seven molecules was selected as the target training set. Among the seven molecules of the training set, **4b** was chosen as reference compound, which were allowed to map all features, and another six molecules were allowed to map partially on the hypotheses for hypoglycemic activity (Table 2). Except for this classification, the activities of the molecules were not used in the analysis. This tool builds hypotheses (overlays common features) for which the fit of individual molecules to a hypothesis can be correlated with the molecule's activity. The 3-D hypothesis study was performed with the Catalyst (version 4.9) package. The geometry of each compound was built with a visualizer and optimized by using the generalized CHARMm-like force field implemented in the program. A preparative test was performed with hydrogen-bond acceptor (HBA), hydrogen-bond acceptor lipid (HBAI), hydrogen-bond donor (HBD), hydrophobic (Hp), hydrophobic aromatic (HpAr), hydrophobic aliphatic (HpAl), negative ionizable (NI), positive ionizable (PI), and Ring Aromatic (R) (Greene *et al.*, 1994). NI and PI were used rather than negative charge and positive charge to broaden the search for deprotonated and protonated atoms or groups at physiological pH. Using conformational poling (Smellie *et al.*, 1994), a representative family of conformers was generated, within a 20 kcal/mol range of the computed minimum, for each molecule. Potential hypothesis models were produced with the minimum permitted interfeature spacing of 2.00 Å generating alignments of common features (Barnum *et al.*, 1996), which included the projected point of HpAr and HBA (Greene *et al.*, 1994).

Table 1 Training set of compounds tested for hypoglycemic activity



Comp. No	Y	X	R	Z	Insulin release (%)[a]
1a	S	O	H	Single	135.5 ± 16.60 (4)
2a	S	O	H	Double	55.70 ± 9.51 (3)
3a	S	O	CH_3	Double	145.1 ± 18.30 (4)
4a	S	O	C_2H_5	Double	117.9 ± 19.40 (4)
5a	NH	O	H	Double	105.1 ± 7.47 (4)
6a	NCH_3	O	CH_3	Double	103.4 ± 18.66 (3)
7a	NC_2H_5	O	C_2H_5	Double	127.1 ± 5.767 (3)
8a	NH	S	H	Double	130.3 ± 4.04 (4)
9a	NCH_3	S	CH_3	Double	114.3 ± 6.32 (3)
10a	NC_2H_5	S	C_2H_5	Double	96.27 ± 5.78 (3)
Glucose [3.0 mmol/l]					62.96 ± 5.68 (9)
Glucose [5.6 mmol/l]					100.0 (9)
Glibenclamide [1 μg/ml]					210.4 ± 15.6 (11)
Rosiglitazone [10 μmol/l]					116.0 ± 3.0 (8)



Comp. No	Y	X	R	Z	Insulin release (%)[a]
1b	S	O	H	Single	69.89 ± 6.237 (3)
2b	S	O	H	Double	79.76 ± 13.11 (3)
3b	S	O	CH_3	Double	134.9 ± 20.55 (3)
4b	S	O	C_2H_5	Double	299.0 ± 5.572 (3)
5b	NH	O	H	Double	103.3 ± 13.54 (3)
6b	NCH_3	O	CH_3	Double	218.3 ± 15.94 (3)
7b	NH	O	C_2H_5	Double	167.8 ± 17.75 (3)
8b	NH	S	H	Double	247.1 ± 54.66 (3)
9b	NCH_3	S	CH_3	Double	102.8 ± 26.33 (3)
10b	NH	S	C_2H_5	Double	129.6 ± 21.60 (3)

Table 1 continued

Comp. No	Y	X	R	Z	Insulin release (%)[a]
Glucose [3.0 mmol/l]					69.70 ± 4.98 (8)
Glucose [5.6 mmol/l]					100.0 (8)
Glibenclamide [1 μg/ml]					250.6 ± 18.10 (3)
Rosiglitazone [10 μmol/l]					116.0 ± 3.0 (8)

[a] Effects of various compounds on glucose-mediated insulin release from INS-1 cells. INS-1 cells in multi-wells were washed three times and incubated in K R BH-buffer for 90 min at 5.6 mmol/l glucose alone. Values obtained in the presence of 3.0 mmol/l glucose (substimulatory concentration) and glibenclamid (1 μg/ml) served as controls. Each value represents the mean ± SEM, number of independent experiments in parentheses

Table 2 Characteristic for the common feature hypothesis run

Compound	Confs[a]	Features/confs[a]	Principal[b]	MaxOmitFeat[c]
3a	11	11.82	1	2
8a	11	11.27	1	2
3b	29	11.52	1	2
4b	64	11.59	2	0
6b	33	11.09	1	2
7b	59	11.10	1	2
8b	40	10.25	1	2

[a] *Confs* number of conformers, *Features/Confs* total number of features divided by the number of conformers (summed over the entire family of conformers)

[b] Principal = **1** means that this molecule must map onto the hypotheses generated by the search procedure. Partial mapping is allowed. Principal = **2** means that this is a reference compound. The chemical feature space of the conformers of such a compound is used to define the initial set of potential hypotheses

[c] The MaxOmitFeat column specifies how many hypothesis features must map to the chemical features in each compound. A **0** in this column forces mapping of all features, and a **2** allows hypotheses to which no compound features map

It was found that hypotheses contain good correlation with HpAr and HBA. The characteristics of ten hypotheses are listed in Table 3. All the hypotheses contain six features with the ranking scores ranking from 121.554 to 117.847. All hypotheses consist of the same common-feature functions two HpArs and four HBAs. The rank score range over the ten generated hypotheses is 3.707.

Hypothesis 1 has been chosen for further evaluation. Figures 1–3 depict **4b**, the most active compound, **6b** and **8b**, which are analogues of **4b**, mapped onto hypothesis 1, respectively. The molecules **4b** and **6b** map well onto the six features of hypothesis 1 (Figs. 1 and 2), whereas HBA do not map on the oxo group of thiazolidinedione and another HBA slightly map oxo group of benzopyran in **8b** (Fig. 3). It could be considered that the 2,4-thiazolidindione ring was necessary for increasing activity. The compound **3b**, which has 2,4-thiazolidindione ring, maps well onto the two HpArs of this hypothesis but it slightly maps to three HBAs. An

Table 3 Results of the common feature hypothesis run

Hypotheses	Feature[a]	Rank score	Direct hit[b]	Partial hit[b]
1	HpAr HpAr HBA HBA HBA HBA	121.554	1111111	0000000
2	HpAr HpAr HBA HBA HBA HBA	119.824	1111111	0000000
3	HpAr HpAr HBA HBA HBA HBA	119.824	1111111	0000000
4	HpAr HpAr HBA HBA HBA HBA	119.314	1111111	0000000
5	HpAr HpAr HBA HBA HBA HBA	118.919	1111111	0000000
6	HpAr HpAr HBA HBA HBA HBA	118.919	1111111	0000000
7	HpAr HpAr HBA HBA HBA HBA	118.171	1111111	0000000
8	HpAr HpAr HBA HBA HBA HBA	118.171	1111111	0000000
9	HpAr HpAr HBA HBA HBA HBA	117.847	1111111	0000000
10	HpAr HpAr HBA HBA HBA HBA	117.847	1111111	0000000

[a] HpAr = hydrophob aromatic; HBA = hydrogen-bond acceptor

[b] Direct hit, all the features of the hypothesis are mapped. Direct Hit = 1 means yes; Partial Hit, partial mapping of the hypothesis. Partial Hit = 0 means no. Each number refers to a molecule in Table 2 (same order)

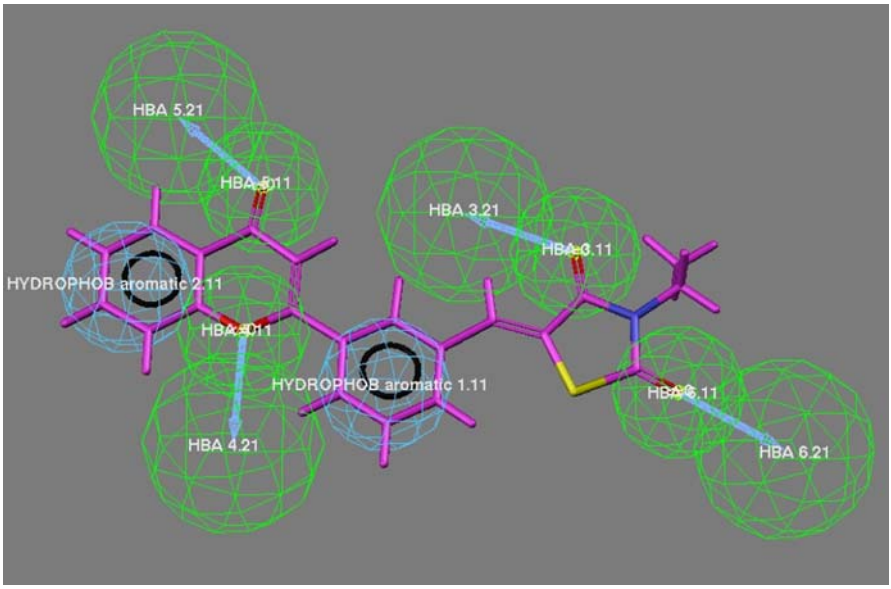


Fig. 1 Mapping of **4b** onto hypothesis 1, which contains two HpArs (*blue*) and four HBAs (*green*). (Color figure online)

idea is that attaching an ethyl group instead of methyl is important for getting better activity. On the other hand, molecule **3a** maps well onto only one feature: HpAr. Another HpAr slightly fit onto phenyl ring. It can be concluded that substituents at 4′ position of phenyl are not favorable.

Molecule **7b** maps to one HpAr and one HBA. It could be pointed out that an alkyl substitutions of both "N" of imidazolidine are very important compared with

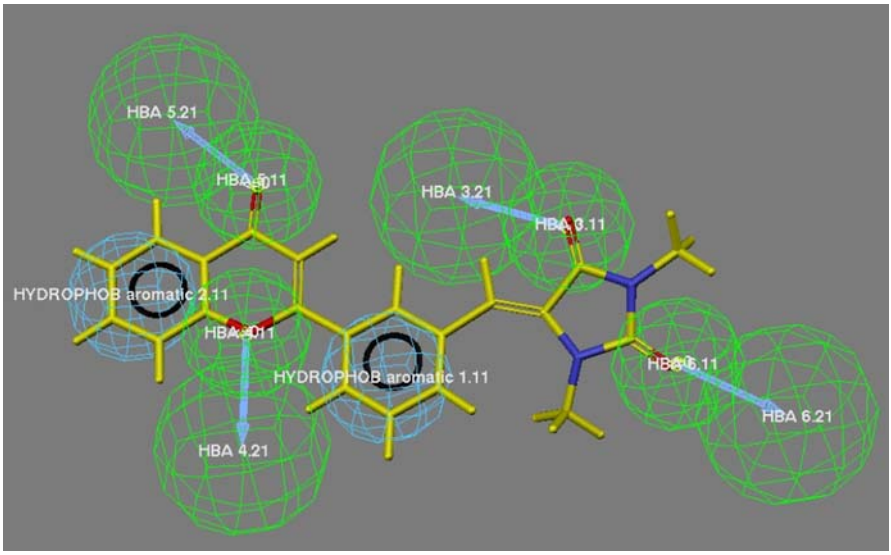


Fig. 2 Mapping of **6b** onto hypothesis 1, which contains two HpArs (*blue*) and four HBAs (*green*). (Color figure online)

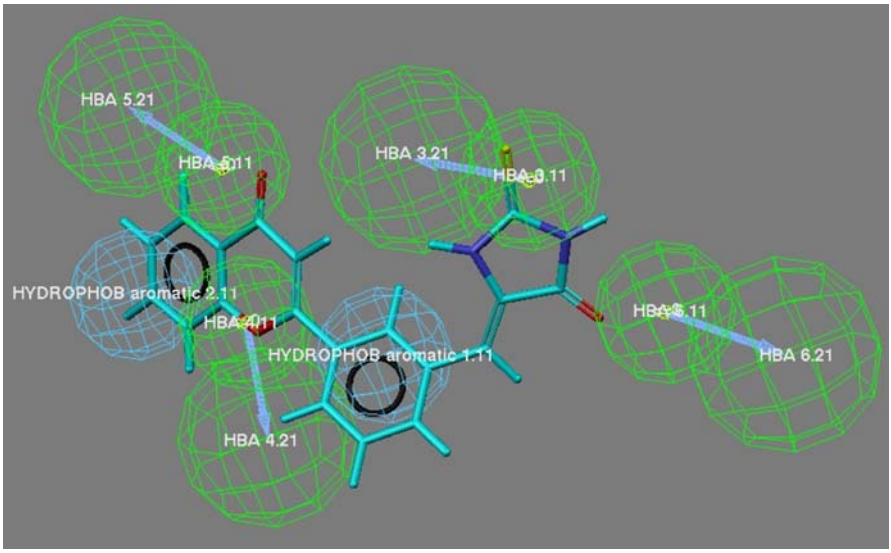


Fig. 3 Mapping of **8b** onto hypothesis 1, which contains two HpArs (*blue*) and four HBAs (*green*). (Color figure online)

compound **6b**. Moreover, the standard structure, glibenclamide, was compared with hypothesis 1 using best fit to validate this hypothesis (Fig. 4). For this reason, a conformation of glibenclamide (15.0644 kcal/mol) map well onto the five features of hypothesis 1.

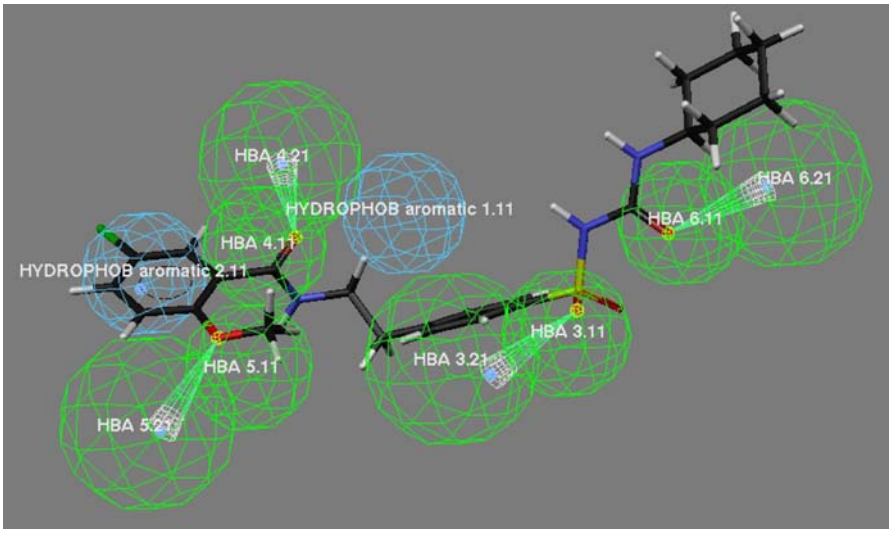


Fig. 4 Mapping of **Glibenclamide** onto hypothesis 1, which contains two HpArs (*blue*) and four HBAs (*green*). (Color figure online)

Conclusions

In rational drug design process, it is common that the biological activity data of a set of compounds acting upon a particular protein is known, whereas information of the 3-D structure of the protein active site is absent (Hirashima *et al.*, 2002). A 3-D pharmacophore hypothesis that is consistent with known data should be useful and predictive in evaluating new compounds and directing further synthesis. A pharmacophore model postulates that there is an essential 3-D arrangement of functional groups that a molecule must possess to be recognized by the active site. It collects common features distributed in 3-D space, which is intended to represent groups in a molecule that participates in important interactions between drugs and their active sites. Hence, a pharmacophore model provides crucial information about how well the common features of a subject molecule overlap with the hypothesis model. It also informs the ability of molecules to adjust their conformations to fit an active site with energetically reasonable conformations. Such characterized 3-D models convey important information in an intuitive manner.

This study shows how a set of activities of hypoglycemic agents may be treated statistically to uncover the molecular characteristics that are essential for high activity. Hypotheses were obtained and applied to map the active or inactive compounds. Significant features, such as two HpArs and four HBAs of the surface-assessable models, were found for hypoglycemic activity. It was found that the most active molecule, 3-ethyl-5-[3′-(4H-4-oxo-1-benzopyran-2-yl)benzylidene]-2,4-thiazolidinedione (**4b**), maps well to all features of the hypotheses. We believe that these observations could be guided for searching new candidate hypoglycemic agents.

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