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# α-Methylation at benzylic fragment of *N*-aryl-*N*′-benzyl ureas provides TRPV1 antagonists with better pharmacokinetic properties and higher efficacy in inflammatory pain model

Arthur Gomtsyan,* Erol K. Bayburt, Ryan Keddy, Sean C. Turner, Tammie K. Jinkerson, Stanley Didomenico, Richard J. Perner, John R. Koenig, Irene Drizin, Heath A. McDonald, Carol S. Surowy, Prisca Honore, Joe Mikusa, Kennan C. Marsh, Jill M. Wetter, Connie R. Faltynek and Chih-Hung Lee

*Global Pharmaceutical Research and Development, Abbott Laboratories, 100 Abbott Park Road, Abbott Park, IL 60064, USA*

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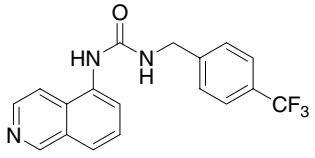
Dedicated to Professor Ivars Kalvinsh on the occasion of his 60th birthday.

**Abstract**—SAR studies for *N*-aryl-*N*′-benzyl urea class of TRPV1 antagonists have been extended to cover α-benzyl alkylation. Alkylated compounds showed weaker in vitro potencies in blocking capsaicin activation of TRPV1 receptor, but possessed improved pharmacokinetic properties. Further structural manipulations that included replacement of isoquinoline core with indazole and isolation of single enantiomer led to TRPV1 antagonists like (*R*)-**16a** with superior pharmacokinetic properties and greater potency in animal model of inflammatory pain.


Transient receptor potential vanilloid 1 (TRPV1) is currently one of the most attractive therapeutic targets in pain research.[1] Nonselective cation channel TRPV1, originally described as a molecular target for capsaicin, pungent component of chili pepper, is now considered to be the principal integrator of noxious information. The reason for such characterization lies in the fact that there are multiple mechanisms by which TRPV1 can be either activated or sensitized. For example, activation of TRPV1 channel can be achieved by vanilloids such as capsaicin and resiniferatoxin, noxious heat (>42 °C), acidic extracellular media (pH < 6), and endogenous anandamide and arachidonic acid metabolites among other agents. On the other hand, activation of TRPV1 is potentiated by prostaglandins, bradykinin, and other pro-inflammatory substances.[2] Blocking the painful effects of activators by TRPV1 antagonists is one of the major avenues in discovery of novel analgesics.

Recently two groups reported on discovery of *N*-isoquinolin-*N*′-benzyl urea class of TRPV1 antagonists.[3,4] This series produced lead compound **1** (Fig. 1) characterized by good potency at TRPV1,[5] modest efficacy in animal pain models[6], and less than desirable pharmacokinetic profile. Here we discuss the effects of alkylation and arylation at the benzylic carbon atom on in vitro, in vivo, and pharmacokinetic properties of TRPV1 antagonists.

General synthetic approach to the synthesis of target TRPV1 antagonists is shown in Scheme 1. Starting arylalkylketones **2** were converted to corresponding oximes **3** which then were reduced to amines **4** and further




**1**

**Figure 1.** Lead TRPV1 antagonist **1**.

*Keywords*: TRPV1; TRPV1 antagonist; Urea; Pain.
* Corresponding author. Tel.: +1 847 935 4214; fax: +1 847 937 9195; e-mail: arthur.r.gomtsyan@abbott.com

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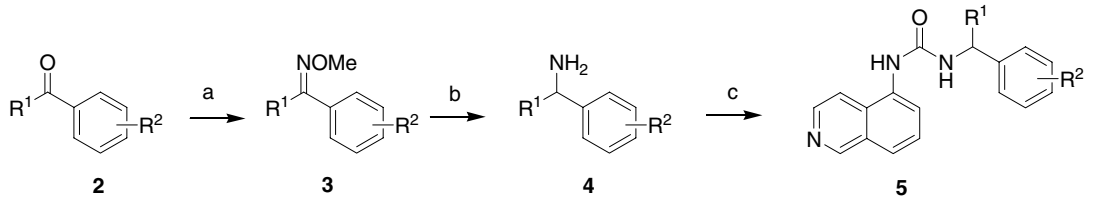
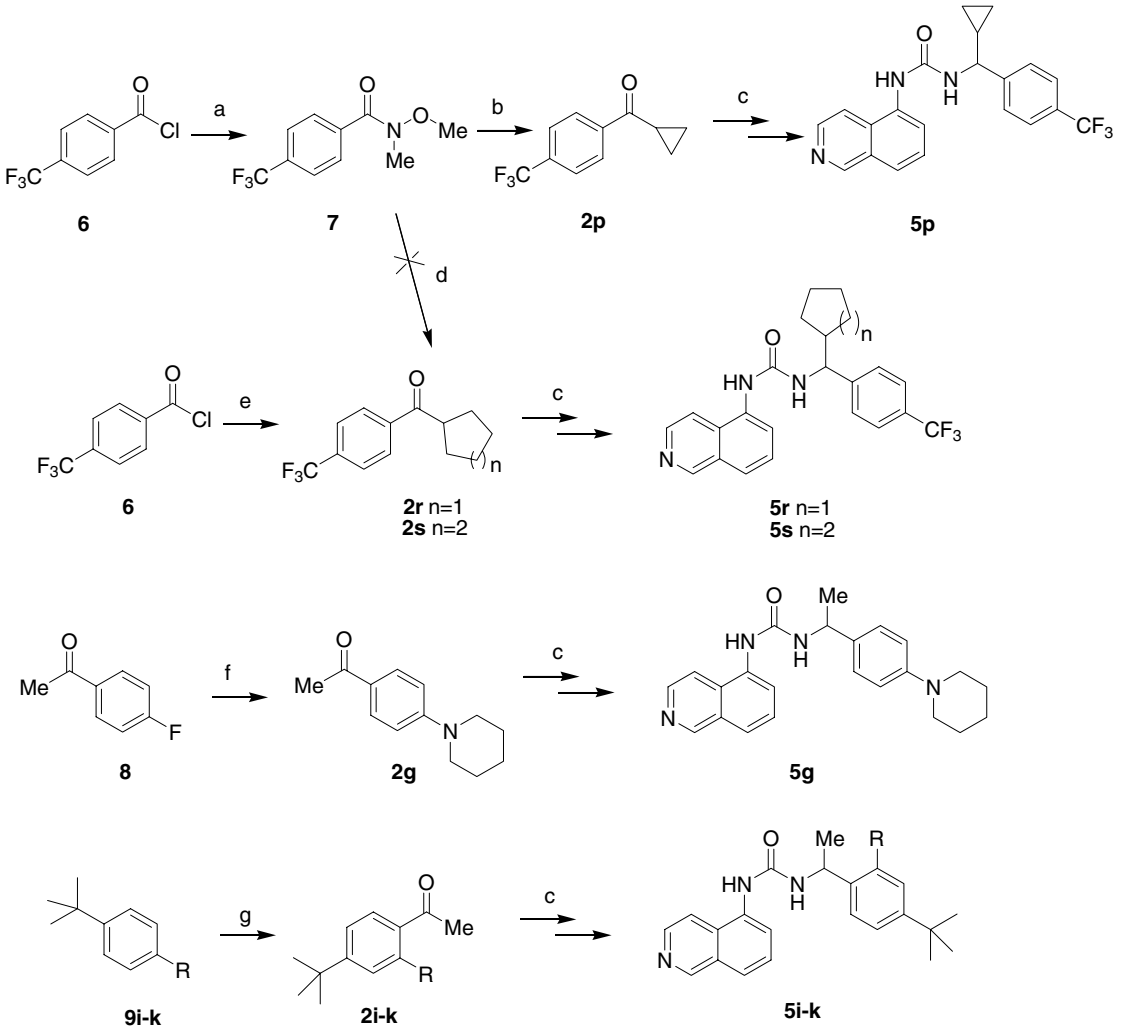
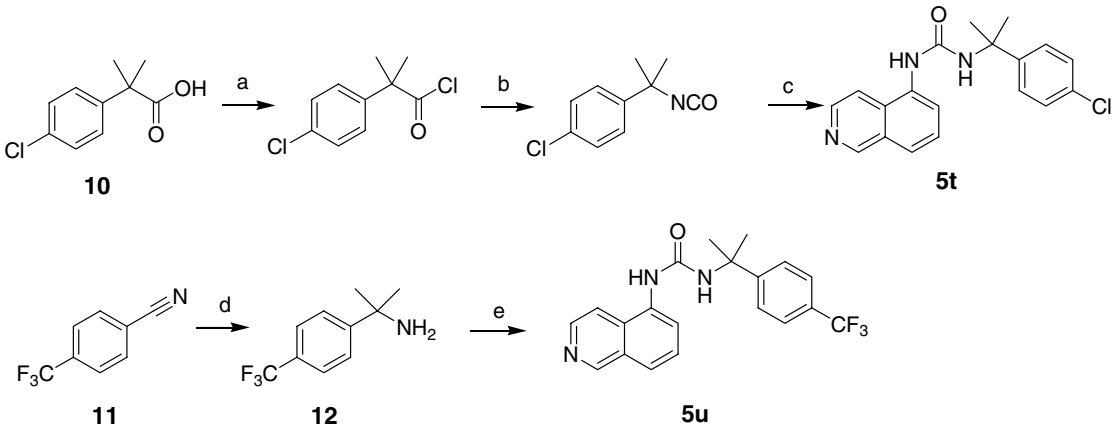


**Scheme 1.** Reagents and conditions: (a) $H_2NOMe{\cdot}HCl$, Py, 16 h, rt; (b) $H_2$, 10% Pd/C, MeOH–$NH_3$; (c) 5-isocyanatoisoquinoline, $CH_2Cl_2$, rt or 2,2,2-trichloro-*N*-(isoquinolin-5-yl)acetamide, MeCN, DBU, reflux.

elaborated to desired compounds **5** by reacting with isoquinoline isocyanate or its surrogate trichloroacetamide.[3] Scheme 2 outlines synthetic detours necessary to prepare commercially unavailable starting ketones **2**. Thus, for the synthesis of cyclopropyl-containing ketone **2p**, corresponding Weinreb amide **7** was prepared from 4-trifluorobenzoyl chloride (**6**) and then reacted with cyclopropylmagnesium bromide. Under the same reaction conditions cyclopentylmagnesium bromide yielded only traces of the corresponding ketone **2r**. However, reactions between acid chloride **6** and cyclopentyl- and cyclohexylzinc-bromides were more successful providing desired ketones **2r** and **2s** in 52 and 59% yields, respectively. 4-Piperidinophenyl-methyl ketone (**2g**) was prepared in one step from corresponding fluoro-substituted ketone **8** by refluxing the mixture of reactants in pyridine. For the synthesis of compounds **5i**–**k** where phenyl group in the benzyl moiety contains two substituents one of which is *tert*-butyl, we took advantage of known phenomenon of *tert*-butyl



**Scheme 2.** Reagents and conditions: (a) NH(OMe)Me·HCl, Py, THF, 0 °C, rt, 2 h, quantitative; (b) cyclopropylmagnesium bromide, THF, 0 °C, 1 h, 48%; (c) see Scheme 1, a–c; (d) cyclopentylmagnesium bromide, THF, 0 °C, 2 h; (e) cycloalkylzinc bromide, CuCN, LiBr, THF, −45 °C, rt, 52% for **2r** and 59% for **2s**; (f) piperidine, Py, reflux, 2 h, 86%; (g) $AlCl_3$, $CS_2$, then MeCOCl, 49–95%.

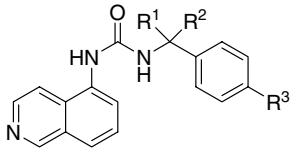



**Scheme 3.** Reagents and conditions: (a) $SOCl_2$, toluene, 80 °C, 2 h, 98%; (b) →(i→) $NaN_3$, $H_2O$, 0 °C, 0.5 h;→ (ii→)toluene, 60 °C, 1 h, 96% for two steps; (c) 5-aminoisoquinoline, THF, 16 h, rt, 38%; (d) methylmagnesium bromide, THF, 0 °C, rt, 16 h; (e) see Scheme 1c.

isomerization/migration that occurs during electrophilic reactions on *tert*-butyl-substituted benzenes.[7] Thus, Friedel–Crafts acetylation of disubstituted benzene **9i–k** in carbon disulfide in the presence of aluminum chloride initiated *tert*-butyl group migration from *para*- to *meta*-position and gave desired ketones **2i–k** in good yields. For the synthesis of gem-dimethyl compounds **5t** and **5u** two approaches were taken (Scheme 3). Curtius rearrangement strategy starting from the carboxylic acid **10** led to **5t** and reduction of nitrile **11** to amine **12** was the key reaction[8] in the preparation of **5u**.

Inspection of SAR Table 1 reveals that attachment of the methyl group to the benzylic carbon atom provides superior TRPV1 antagonists compared with the ethyl (**5d** vs **5l**), phenyl (**5a** vs **5e**), cycloalkyl (**5a** vs **5p–s**) or gem-dimethyl (**5a** vs **5u**) analogs. Although in vitro activities of the most potent methyl-substituted compounds **5a–d**, **j**, **k** were still 4- to 5-fold weaker than for unsubstituted **1**, we found that methylation at the benzylic carbon atom led to TRPV1 antagonists exhibiting longer half-life and larger volume of distribution in both rat and dog pharmacokinetic studies (Table 2).

**Table 1.** In vitro functional activity of isoquinoline TRPV1 antagonists **5** in human TRPV1 $Ca^{2+}$ influx assay

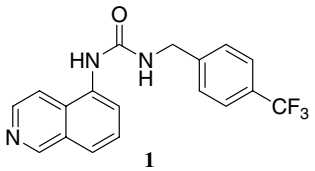
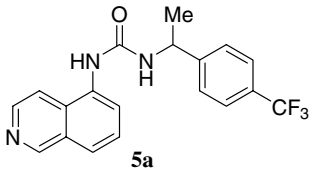



**5**

| Compound | $R^1$ | $R^2$ | $R^3$ | hTRPV1 $IC_{50}$[a] (nM) |
|---|---|---|---|---|
| **1** | H | H | 4-$CF_3$ | 4 ± 3 |
| **5a** | Me | H | 4-$CF_3$ | 22 ± 9 |
| **5b** | Me | H | 4-Br | 26 ± 10 |
| **5c** | Me | H | 4-*t*-Bu | 24 ± 4 |
| **5d** | Me | H | 3-F, 4-$CF_3$ | 19 ± 5 |
| **5e** | Ph | H | 4-$CF_3$ | 147 ± 21 |
| **5f** | Ph | H | 4-*t*-Bu | 125 ± 30 |
| **5g** | Ph | H | 4-Piperidino | 171 ± 52 |
| **5h** | Ph | H | H | 600 ± 0[b] |
| **5i** | Me | H | 2-Me, 4-*t*-Bu | 46 ± 12 |
| **5j** | Me | H | 2-Et, 4-*t*-Bu | 20 ± 5 |
| **5k** | Me | H | 2,4-di-*t*-Bu | 17 ± 5 |
| **5l** | Et | H | 3-F, 4-$CF_3$ | 109 ± 11 |
| **5m** | Et | H | H | 2580 ± 350 |
| **5n** | $Me_2NCH_2$ | H | H | 31,000 ± 2200 |
| **5o** | $NCCH_2$ | H | H | 15,900 ± 1100 |
| **5p** | Cyclopropyl | H | 4-$CF_3$ | 149 ± 50 |
| **5r** | Cyclopentyl | H | 4-$CF_3$ | 476 ± 180 |
| **5s** | Cyclohexyl | H | 4-$CF_3$ | 287 ± 118 |
| **5t** | Me | Me | 4-Cl | 143 ± 29 |
| **5u** | Me | Me | 4-$CF_3$ | 94 ± 14 |

[a] All values are means ± SEM of at least three separate experiments.
[b] Only one reading.

**Table 2.** Pharmacokinetic properties of *N*-benzyl-*N*′-isoquinolinyl urea **1** and its methylated analog **5a**[a]

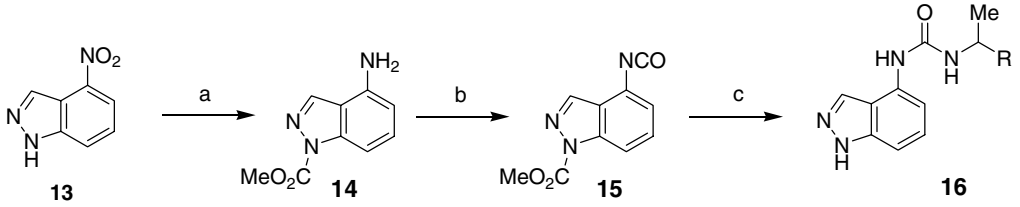
| Compound | hTRPV1 $IC_{50}$ (nM) | iv $T_{1/2}$ (h) | iv $V_\beta$ (L/kg) | iv Clp (L/h kg) | po *F* (%) |
|---|---|---|---|---|---|
|  **1** | 4 | 0.6 (rat)<br>1.0 (dog) | 0.6 (rat)<br>0.5 (dog) | 0.6 (rat)<br>0.4 (dog) | 46 (rat)<br>32 (dog) |
|  **5a** | 22 | 1.1 (rat)<br>1.6 (dog) | 1.1 (rat)<br>1.1 (dog) | 0.7 (rat)<br>0.5 (dog) | 47 (rat)<br>98 (dog) |

[a] Pharmacokinetic parameters determined in rats and dogs following administration of 10 μmol/kg.

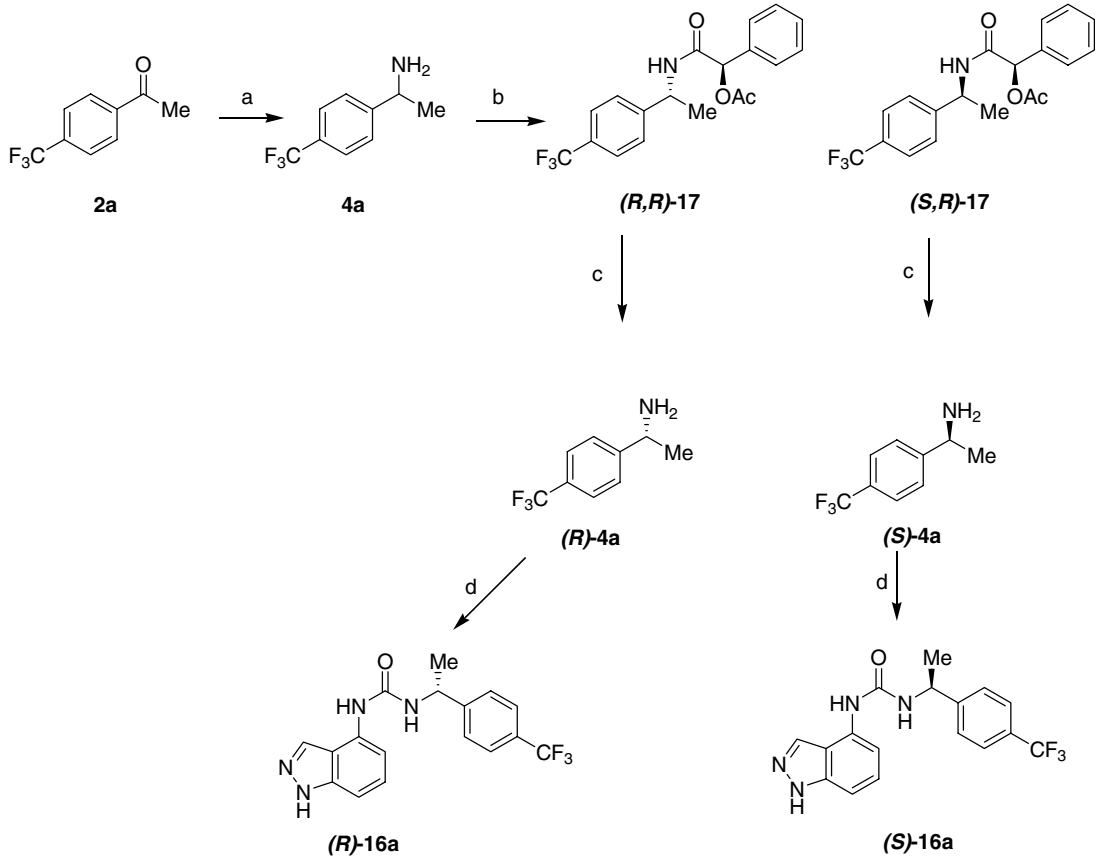
In an effort to further improve pharmacokinetic properties of target molecules, we replaced the isoquinoline with an indazole moiety that was previously used in the unsubstituted series.[9] Thus, 4-nitroindazole (**13**)[10] was acylated by methylchloroformate followed by catalytic hydrogenation over Pd/C to provide 4-aminoindazole derivative **14** which was converted to isocyanate intermediate **15**[9] (Scheme 4). The latter reacted with several α-methyl-benzylamines to afford corresponding ureas, which after indazole N-deprotection by NaOH in MeOH gave target compounds **16**. In order to resolve individual enantiomers of **16a**, several approaches were taken such as (1) asymmetric synthesis of the chiral amines where the key reaction was chiral oxazaborolidine catalyzed reduction of acetophenones to secondary alcohols[11,12] followed by elaboration to amines; (2) chiral resolution of racemic amines either by chiral acids or diastereomer formation; (3) HPLC separation of final racemic products on chiral columns. The most useful and reproducible results were obtained by chiral resolution of racemic amine **4a** through diastereomer formation (Scheme 5). Thus, acetophenone **2a** was converted to racemic amine **4a** in two steps consisting of oxime formation followed by reduction. Then, the diastereomeric mixture of amides **17**, prepared from **4** and (*R*)-*O*-acetylmandelic acid, was separated on silica gel to give diastereomerically pure amides (*R*,*R*)-**17** and (*S*,*R*)-**17** in 42 and 39% yields, respectively. Finally, the cleavage of the chiral auxiliary by treating the amides with aq HBr afforded desired chiral amines (*R*)-**4a** and (*S*)-**4a** in good yields. Absolute stereochemistry was assigned based on the sign of reported optical rotation.[13] NMR and HPLC analysis of Mosher amides of synthesized amines confirmed enantiomeric ratios of 96:4 and 97:3 for (*R*)-**4a** and (*S*)-**4a**, respectively. Synthesis of target chiral compounds (*R*)-**16a** and (*S*)-**16a** was completed in two steps from amines (*R*)-**4a** and (*S*)-**4a** by their acylation with isocyanate **15** followed by deprotection of methoxycarbonyl group. Commercially available chiral amines were used for the synthesis of single enantiomers of **16b**,**c**.

Appraisal of enantiomeric pairs of TRPV1 antagonists **16** in indazole series indicated clear effect of chirality on the functional potency of TRPV1 antagonists in the $Ca^{2+}$ influx assay (Table 3). (*R*)-Enantiomers of **16a**–**c** were ~5- to 30-fold more potent than their (*S*)-counterparts.

The most potent compound from these series, (*R*)-**16a**, was tested in rat model of inflammatory pain and the result was compared with the activity of compound **1** in the same model. Compound (*R*)-**16a** demonstrated greater potency in vivo than **1** in relieving CFA (Complete Freund's Adjuvant)-induced thermal hyperalgesia[14] after oral administration (Table 4), despite the fact that it was 10-fold weaker than **1** in $Ca^{2+}$ influx assay. The lack of correlation between in vitro and in vivo activities for **1** and (*R*)-**16a** can be explained by better pharmacokinetic properties of (*R*)-**16a** compared with **1**. The most notable differences were larger volume of distribution (2.9 vs 0.6 L/kg) and longer half-life (2.1 vs 0.6 h) for (*R*)-**16a**.

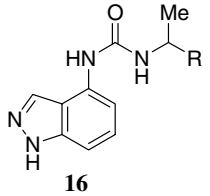



**Scheme 4.** Reagents and conditions; (a)→ (i→) mix NaH (60% in mineral oil) and DMF, add **13** at 0 °C, then methylchloroformate, 0 °C, rt, 51%;→ (ii→) $H_2$, 10% Pd/C, MeOH, 60 psi, 60 °C, 1 h, used crude; (b) 20% $COCl_2$ in toluene, reflux, 3 h, used crude; (c)→ (i→) benzylamines, $Et_2O$, $Et_3N$, 16 h, rt; →(ii→) 5 M NaOH in MeOH, 2 h, rt.




**Scheme 5.** Reagents and conditions: (a) see Scheme 1, a and b; (b) →(i→) (*R*)-*O*-acetylmandelic acid, DCC, DMAP, $CH_2Cl_2$, rt, 16 h; →(ii→) silica gel chromatography, 42% for (*R*,*R*)-**17** and 39% for (*S*,*R*)-**17**; (c) 48% HBr (aq), $H_2O$, reflux, 75% for (*R*)-**4a** and 72% for (*S*)-**4a**; (d) see Scheme 4, c.

**Table 3.** In vitro functional activity of indazole TRPV1 antagonists **16** in human TRPV1 $Ca^{2+}$ influx assay




| Compound | R | hTRPV1 $IC_{50}$[a] (nM) |
|---|---|---|
| (*rac*)-**16a** | 4-$CF_3$-phenyl | 47 ± 11 |
| (*S*)-**16a** | 4-$CF_3$-phenyl | 248 ± 62 |
| (*R*)-**16a** | 4-$CF_3$-phenyl | 41 ± 10 |
| (*S*)-**16b** | 4-Me-phenyl | 749 ± 115 |
| (*R*)-**16b** | 4-Me-phenyl | 73 ± 5 |
| (*S*)-**16c** | naphthyl | 3750 ± 1900 |
| (*R*)-**16c** | naphthyl | 123 ± 7 |

[a] All values are means ± SEM of at least three separate experiments.

**Table 4.** Comparison of in vitro, in vivo, and pharmacokinetic properties of compounds **1** and (*R*)-**16a**

| Compound | hTRPV1 $IC_{50}$ (nM) | CFA $ED_{50}$ (μmol/kg) po[a] | iv $T_{1/2}$ (h)[b] | iv $V_\beta$ (L/kg)[b] | po $F$[b] (%) |
|---|---|---|---|---|---|
| **1** | 4 | 40 | 0.6 | 0.6 | 46 |
| (*R*)-**16a** | 41 | 13 | 2.1 | 2.9 | 53 |

[a] The data represent means of $n = 6$ per dose group.
[b] Pharmacokinetic parameters determined in rats following administration of 10 μmol/kg.

In conclusion, several modifications to the early pharmacological tool compound **1** were made with the goal of improving both pharmacokinetic properties and in vivo activity. These modifications included methylation at benzylic carbon atom, replacement of isoquinoline core with indazole, and finally resolving individual enantiomers. While this process resulted in compounds showing weaker activity at TRPV1, their improved pharmacokinetic properties resulted in greater potency in an animal pain model. The current data also demonstrated that introduction of a stereogenic center may offer additional opportunities to improve pharmacological and pharmacokinetic properties of TRPV1 compounds by virtue of one of the enantiomers being superior to the other.

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