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Discovery of a Series of Indazole TRPA1 Antagonists

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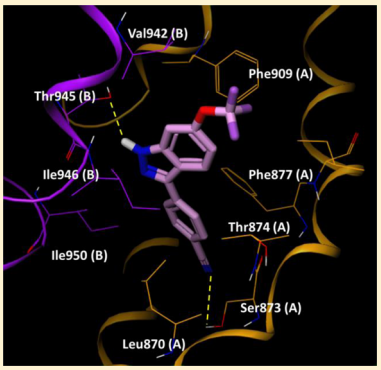
Supporting Information

ABSTRACT: A series of TRPA1 antagonists is described which has as its core structure an indazole moiety. The physical properties and *in vitro* DMPK profiles are discussed. Good *in vivo* exposure was obtained with several analogs, allowing efficacy to be assessed in rodent models of inflammatory pain. Two compounds showed significant activity in these models when administered either systemically or topically. Protein chimeras were constructed to indicate compounds from the series bound in the S5 region of the channel, and a computational docking model was used to propose a binding mode for example compounds.



KEYWORDS: *TRPA1, Transient receptor potential, Pain, Ion channel, Indazole, Cinnamaldehyde flare, AITC, Topical administration*

The transient receptor potential (TRP) family of ion channels function as sensors of multiple chemical and physical stimuli (temperature, smell, taste, and noxious chemicals).[1,2] The TRPA1 (transient receptor potential ankyrin-repeat 1) channel is a nonselective cation channel that is implicated in many aspects of sensation, including pain and thermosensation.[3] TRPA1 is activated by a variety of ligands, including exogenous electrophiles, such as cinnamaldehyde, acrolein, allyl isothiocyanate (AITC), and the endogenous ligand 4-hydroxynonenal. Recombinant TRPA1 is activated by noxious cold (<17 °C). Electrophilic ligands have been shown to activate TRPA1 though covalent binding to cysteine residues present in the cytoplasmic N-terminus of TRPA1 in *in vitro* systems.[4] When activated, TRPA1 permits the conduction of sodium and calcium ions from the extracellular environment into the cell, depolarizing the membrane and affecting calcium homeostasis in the primary afferents. Depolarization of the primary nerve terminals leads to action potential firing and consequently increased pain sensation and hyperalgesia in man.[5]

The TRPA1 channel has been directly linked to pain in humans by a gain-of-function mutation that causes familial episodic pain syndrome.[6] TRPA1 antagonists have also been shown to reverse pain in various rodent models.[7] These and related data have stimulated significant interest in the biomedical industry to seek potent and selective TRPA1 antagonists. A large number of disclosures have been made of TRPA1 chemotypes from across the industry. A selection of these, **1**−**7**, are illustrated in Figure 1, taken from patent publications and the journal literature.[2,3,5] Hydra Biosciences, in partnership with Cubist Pharmaceuticals, recently advanced a TRPA1 antagonist CB-189625 into a nociceptive pain Phase 1 clinical trial.[8,9] Hydra have also advanced another compound, HX-100, into trials of painful diabetic neuropathy and allergic asthma.[10] Glenmark Pharmaceuticals has reported positive data in a diabetic peripheral neuropathy Phase 2 study with GRC17536 in patients with neuropathic pain and asthma.[11−13] The structures of these compounds have not been disclosed as yet, but they have been described in the literature as very potent and selective TRPA1 antagonists.

Our program started with a high-throughput screen of the internal compound library using an antagonist mode FLIPR Ca^{2+} imaging assay in 384-well format. All hits were confirmed in a single rig electrophysiology (EP) assay. The hit rate from this screen was low but provided compound **8** as an initial hit (Figure 2). **8** was a quite lipophilic, weak base of moderate potency, which was confirmed in a manual patch (MP) clamp EP assay to have an IC_{50} of 0.55 μM and low LipE. Upon further investigation, **8** was found to have some affinity for other ion channels, most notably the KCNQ2/3 channel with

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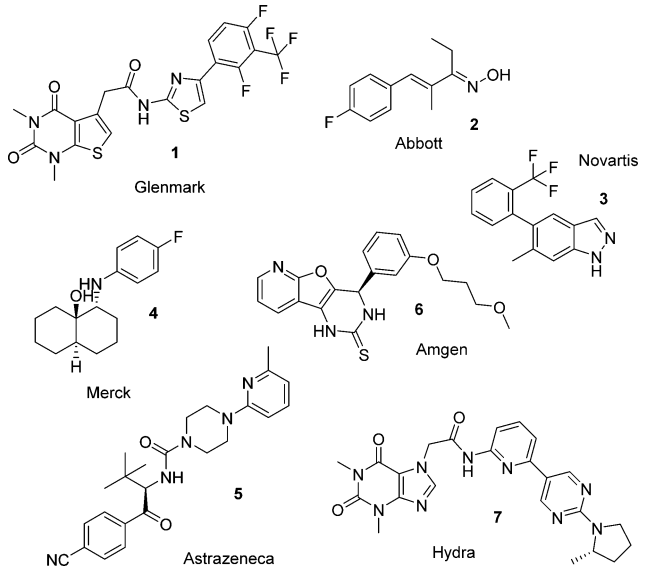
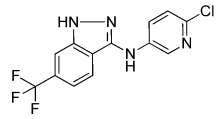


Figure 1. Examples of chemotypes with reported TRPA1 activity.



8
TRPA1 hFLIPR IC_{50} = 2.5 μM
hEP IC_{50} = 0.55 μM (LipE 1.6)
cLogP 4.7, MWt. 313

hERG IC_{50} >100μM
KCNQ1 IC_{50} 2640nM
KCNQ2/3 EC_{50} 37nM
Nav1.5 IC_{50} 8520nM
TRPV1 IC_{50} >30μM
SK2 EC_{50} >10μM

Figure 2. File screening hit.

an activation EC_{50} of 37 nM and the KCNQ1 channel with an inhibition IC_{50} of 2.6 μM,[14] although no activity at the TRPV1 channel was observed.

It is notable that a recent report has described a series of compounds of similar structure (e.g., compound **3**) from a Novartis group.[15] At this stage, we wished to explore the structure activity relationships of this series more fully, to understand the structural basis of its TRPA1 potency. The initial FLIPR screen was valuable for hit identification and triage of focused libraries. However, EP was found to be more reliable, and the development of directed compound designs was driven by EP potency. Compounds were assayed in a medium throughput EP assay using the PatchXpress (PX) platform with a HEK293-T-rex human TRPA1-expressing cell line. Selected compounds were also tested in a rat cell line, and the most advanced tool compounds were further tested in MP assays.

The first analogues explored were variations of the amino substituent of **8** (Table 1). Larger substituents such as the CF_3-pyridine **9** were substantially weaker than the starting Cl substituent, whereas the equivalent phenyl group in **10** was >10-fold more active than the pyridine derivative, albeit in a more lipophilic structure with similar LipE to **8**. Capping of the hydrogen bond donating groups either individually (**11** and **12**) or together (**13**) lost potency, as did removing the indazole ring substituent (**14**). Alternative halogen substituents on the aniline group, such as the 3,4-diF group (**15**), were equivalent to Cl. Similarly, Cl (**16**) and OCF_3 (**17**) were found to be equivalent substituents to a CF_3 at the indazole 6-position, but larger groups, as in **18** and **19**, were weaker.

Insertion of a nitrogen atom was very well tolerated at the indazole 4-position (**20**), but less so at the 5-position (**21**).

We combined our findings from this phase of the project into a small number of targets, and found that the amino indazole **22** was now reaching very potent levels of TRPA1 inhibition, albeit of high LogP. Further attempts to reduce lipophilicity, for example through nitrogen insertion at the 2-position of the pyridine ring (**23**), were unsuccessful. It should be noted that, for those examples for which rat activity was tested, rat and human potencies were typically within 10-fold of each other.

In a parallel effort, we also explored replacing the amino substituent (Table 2).

Simply removing the amino group produced a significant drop in potency (**24**), but inserting a nitrogen atom at the indazole 4-position allowed a modest recovery in activity (**25**). It was found that OCF_3 versions such as **26** were of very similar TRPA1 activity and the tolerance of 6-substituents on the indazole ring was broadly similar to the amino series, with large (or more polar/charged) groups such as those in **27** and **28** less well tolerated.

An extended aryl group at the 3-position (**29**) was somewhat tolerated, particularly when combined with a 4-aza substitution (**30**). Alternative aryl groups such as a thiophene (**31**) were potent, but once again, polarity as in the pyridine **32** was weaker than the phenyl equivalents (**33** and **34**).

We therefore had a range of compounds that were either highly potent TRPA1 antagonists but were lipophilic, or examples of more polar compounds that had much weaker primary pharmacology. Examples of two compounds that possessed the former more lipophilic profile are illustrated in Table 3, in which we evaluated physicochemical and biopharmaceutic properties more fully.

Both compounds were of low aqueous solubility and modest passive permeability, but we noted that the aryl-indazole **33** was considerably more stable in human hepatic microsomes than the amino derivative **22** (Figure 3).

We further profiled both compounds for activity against other ion channels, in particular against the KCNQ channels that the original hit **8** displayed. **22** retained KCNQ activity, while the aryl-indazole **33** had ablated this off-target activity completely. Interestingly, the latter compound was very weak in the FLIPR assay, highlighting the importance of using EP platforms for SAR development within this chemotype.

The series had therefore provided good TRPA1 potency and in the aryl-indazole series selective inhibition, but physicochemical properties were not ideal for an oral indication. We next explored the likely binding site for the series, and explored ways to evaluate the *in vivo* efficacy of the tool compounds.

To establish the binding site of compounds such as **22** and **33**, TRPA1 species ortholog chimeras using opossum TRPA1 were generated to identify the critical regions of interaction, as described previously.[17] **22** was >1000× more potent against hTRPA1 (IC_{50} = 20 nM) compared to oTRPA1 (IC_{50} > 30 μM). A 27 amino acid sequence within the S5 helix was swapped and **22** potency tested. The potency of the compound positively correlated with the species source of this 27 amino acid S5 region. Gain of potency was observed when the S5 region of human TRPA1 was swapped into oTRPA1 (hS5, IC_{50} = 132 nM). Loss of function was observed in the counterpart chimera (oS5, IC_{50} = 16 μM). Taken together, these data suggest the activity of **22** at TRPA1 is dependent on the S5 helix.

We then sought to provide more granularity of critical residues of interaction using computational docking. Specific residues in the S5 region have been suggested to be involved in interactions with small molecule TRPA1 modulators. Xiao et al.[18] has suggested that Ser873 and Thr874 in human TRPA1

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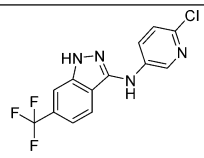
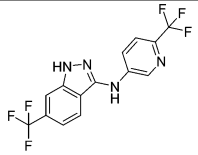
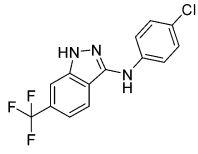
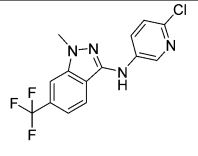
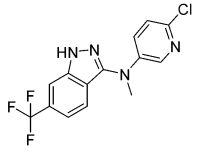
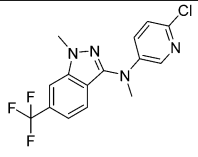
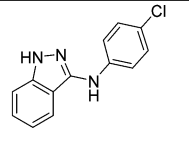
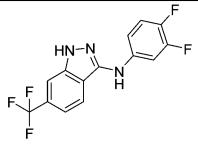
Table 1. Amino-indazole Analogues of Screening Hit 8

Comp	Structure	MWt. (cLogP)	Human TRPA1 PX est. IC50 (nM)	Rat TRPA1 PX est. IC50 (nM)
8		313 (4.7)	546	ND
9		346 (4.9)	>10,000	ND
10		312 (5.9)	42	9
11		327 (4.4)	>10,000	ND
12		327 (4.7)	>10,000	ND
13		341 (4.4)	>10,000	ND
14		244 (4.8)	11,700	ND
15		313 (5.4)	53	9

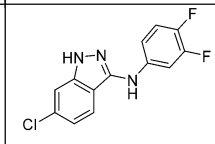
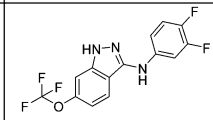
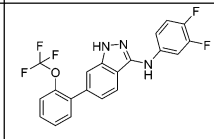
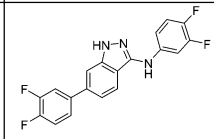
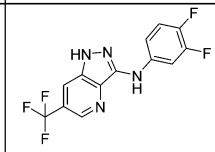
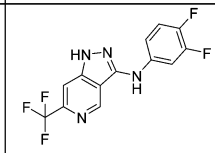
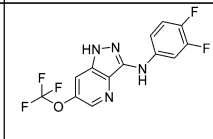
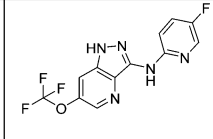
Comp	Structure	MWt. (cLogP)	Human TRPA1 PX est. IC50 (nM)	Rat TRPA1 PX est. IC50 (nM)
16		280 (5.2)	111	49
17		329 (5.5)	131	<100nM 96% @ 1μM
18		405 (6.7)	1203	ND
19		357 (6.5)	3700	ND
20		314 (4.8)	38	23
21		314 (4.6)	645	ND
22		330 (5.3)	20	11
23		313 (4.0)	2537	813

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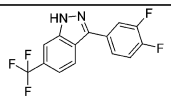
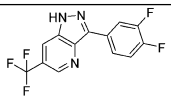
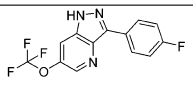
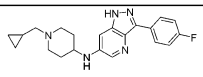
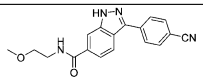
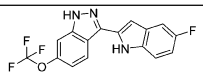
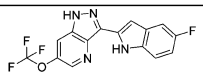
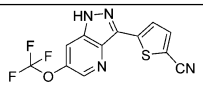
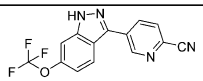
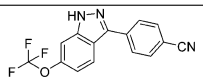
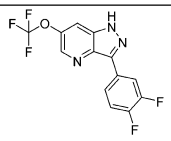
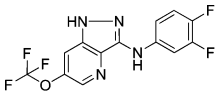
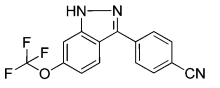
Table 2. Aryl-indazole Analogues of Screening Hit 8

Comp	Structure	MWt (cLogP)	Human TRPA1 PX est. IC50 (nM)	Rat TRPA1 PX est. IC50 (nM)
24		298 (5.1)	2100	329
25		299 (4.4)	450	255
26		297 (4.8)	257	216
27		365 (4.8)	4630	1851
28		320 (2.8)	2667	453
29		335 (5.7)	748	116
30		336 (5.2)	91	8
31		309 (4.5)	74	80
32		304 (3.6)	1182	271
33		303 (4.6)	686	246
34		315 (4.9)	134	128



22
TRPA1 hFLIPR IC_{50} = 0.33 μM
hEP IC_{50} = 0.02 μM (LipE 3.1)

hERG IC_{50} >100μM
KCNQ1 IC_{50} 2640nM
KCNQ2/3 EC_{50} 37nM
Nav1.5 IC_{50} 18200nM
Nav1.7 IC_{50} 8700nM
TRPV1 IC_{50} >30μM
SK2 EC_{50} >10μM



33
TRPA1 hFLIPR IC_{50} = >10 μM
hEP IC_{50} = 0.686 μM (LipE 1.6)

hERG IC_{50} >30μM
KCNQ1 IC_{50} >3000nM
KCNQ2/3 EC_{50} >30μM
Nav1.5 IC_{50} 17300nM
Nav1.7 IC_{50} 17700nM
TRPV1 IC_{50} >30μM
SK2 EC_{50} >10μM

Figure 3. Example tool compounds.

are important for small molecule binding.[19] We docked **33** into the cryo-EM structure of human TRPA1 described by Paulsen et al.[20] and focused on the region around Ser873 and Thr874. Figure 4 shows an example binding mode of **33**. Two H-bond interactions stabilize the complex, formed between the

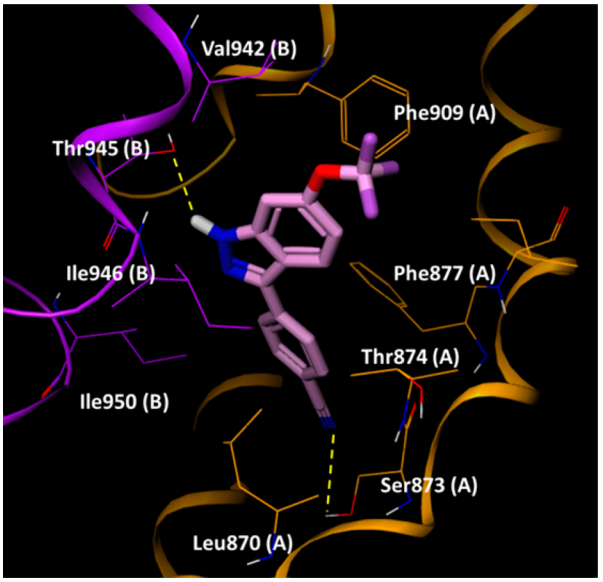


Figure 4. 33 docked into a human TRPA1 construct. Residues from chain A and B are colored orange and purple, respectively, and highlighted residues are suffixed by chain ID for clarity.

OH group of Thr945 and the NH of the indazole, and the other between the CN group and the OH group of Ser873. Thr874 lies close to the indazole 4-position, which could explain the quite specific SAR in this location. 4-Aza substituents resulted in enhancement of human TRPA1 potency, potentially through the OH group of Thr874 forming an H-bond to this N. The 6-position of the indazole was located at the entrance of the binding site. We propose that Val942 in human TRPA1 when mutated to Ile945 in rat TRPA1 supports stronger hydrophobic interactions to extended 6-substituents as in **27** and **28**, which show greater

Table 3. Physicochemical Data of Selected Analogues[a]

Comp	Human TRPA1 PX est. IC_{50} (nM)	HLM/RLM Cl_{int} (μL/min/mg)	cLogP (LogD)	MWt	MDR1 (RRCK) (10^{-6} cm/s)	Aq sol (μg/mL)
22	20	73/99	5.3 (4.6)	330	2/7 (4)	1.6
33	686	<8/58	4.6 (4.6)	303	4/8 (<0.1)	4.8

[a]The metabolism assays are as described.[16] Metabolism data is reported in units of intrinsic clearance. Aqueous solubility was measured from a DMSO stock solution using a kinetic miniaturized shake flask method.

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rat than human potency. Similar docking and binding site analysis for compound **22** also highlighted the same S5 binding region (see Supporting Information), which is very close to the region described by Grandl and co-workers for their indazole series.[15]

The two tool compounds were next evaluated in two models of inflammatory pain. In these studies, we were cognizant of their lipophilicity and low solubility, and therefore that they may be unsuitable as systemic agents. We therefore also wanted to explore the potential of the indazole series to provide a topical agent.

The more metabolically stable **33** was investigated in an allyl isothiocyanate (AITC) model of inflammatory pain (see Supporting Information). After demonstrating similar hTRPA1 and rTRPA1 EP potency, we more rigorously tested rTRPA1 potency in MP using 5 pt concentration−response curves. The PX and MP IC_{50}s were within 2-fold (PX est IC_{50} = 0.246 μM, MP IC_{50} = 0.195 μM), and thus, the MP value was used for subsequent PK/PD calculations. We also extended the selectivity testing of compound **33** to other gene family pain targets in MP. Compound **33** was >100× selective over TRPM8, TRPV3, Nav1.8, and Cav2.2. Upon oral dosing of **33** to rats treated with AITC subcutaneously in the right hind paw, a significant dose-dependent reduction in flinching was observed. **33** showed a minimum effective dose (MED) of 100 mg/kg in this model, which equated to an approximately IC_{50} level of free plasma exposure based on rat MP data.

We also examined the efficacy of **33** in ablating a cinnamaldehyde-induced flare response in the rat using laser Doppler sonography (Figure 5).

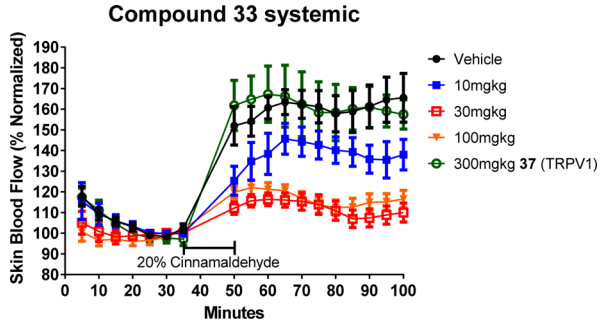

Figure 5. Cinnamaldehyde flare response of systemically applied **33**.

Three oral doses of **33** were investigated: 10, 30, and 100 mg/kg, which achieved mean systemic concentrations of between 11 and 52 nM, below the rat MP *in vitro* IC_{50} (195 nM). An example TRPV1 antagonist **37**,[21−23] was used as a comparative compound of disparate mechanism in the studies, and was not expected to show efficacy in a flare study at a dose that resulted in measured plasma-free exposure of >100× the reported TRPV1 IC_{50} (approximately 3 nM). The laser Doppler data indicated a response at all doses of **33**, which achieved a suppression of the cinnamaldehyde-induced flare response compared to both vehicle and the TRPV1 antagonist. The two higher doses showed a greater response, and overall, the level of response for these relatively low concentrations was very encouraging.

These data provided support for next looking at a topical flare study with the more potent, but less stable **22**. In preparation for this study, we examined the skin flux properties of **22**, which showed low skin permeability (1.2×10^{-5} cm/h) and moderate concentrations both in the epidermal layer (304 μg/g) and in the dermal layer (20 μg/g) following topical administration, confirming the compound had some skin exposure. We also confirmed that **22** had similar MP potency at the rat TRPA1 channel (IC_{50} 11 nM). We designed a topical flare study in the rat, in which **22** was formulated in vehicle, applied to the skin, and left for a defined period of time.[21] A 20% v/v formulation of cinnamaldehyde in the study vehicle was applied to the same location and blood flow then measured in that region by laser Doppler sonography.

The free concentrations achieved with a 50 mg/mL dose in all animals were in the range 7−59 nM (see Supporting Information), similar values to the *in vitro* IC_{50} in both PX and MP (rat MP IC_{50} 11 nM), although presumably the skin concentrations of compound were significantly higher. The suppression of the flare response was very similar to that observed with the systemic application of **33**, clearly showing that a topically administered TRPA1 antagonist can ablate an inflammatory flare response.

In conclusion, we have identified potent and selective inhibitors of the TRPA1 channel based on an indazole template. Efforts to enhance the *in vitro* ADME, off-target pharmacology, and physicochemical properties of this series met with mixed success, but allowed us to identify tool compounds that were used to confirm their region of engagement with the TRPA1 protein. An example compound from the series showed good efficacy in a rat AITC study at approximately IC_{50} levels of compound, and efficacy in a cinnamaldehyde flare study at sub-IC_{50} levels. A further example of the series was examined in a topical flare study and showed efficacy at approximately IC_{50} levels measured systemically. The skin is a major neurosensory organ where the symptoms of pain and itch in chronic diseases are most often first perceived; therefore, we hypothesize that indazole based inhibitors of TRPA1 could offer potential topical treatments for pain or itch.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmedchemlett.7b00140.

Detailed experimental methods, data, synthetic chemistry procedures and additional references are provided as Supporting Information in pdf format. The Supporting Information is available free of charge on the ACS Publications Web site. (PDF)

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Notes

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■ ABBREVIATIONS

TRPA1, transient receptor potential channel A1; AITC, allyl isothiocyanate; EP, electrophysiology; FLIPR, fluorescent imaging plate reader; KCNQ, potassium voltage-gated channel subfamily KQT; MP, manual patch; cryo-EM, cryo-electron microscopy; PX, PatchXpress; ADME, absorption, distribution, metabolism, elimination; MED, minimum effective dose; RRCK, Madin−Darby canine kidney cell line low efflux; MDR1, multidrug resistance gene

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