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Discovery of novel pyrrolopyridazine scaffolds as transient receptor potential vanilloid (TRPV1) antagonists

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ABSTRACT

A novel indolizine class of compounds was identified as TRPV1 antagonist from an HTS campaign. However, this indolizine class proved to be unstable and reacted readily with glutathione when exposed to light and oxygen. Reactivity was reduced by the introduction of a nitrogen atom alpha to the indolizine nitrogen. The pyrrolopyridazine core obtained proved to be inert to the action of light and oxygen. The synthesis route followed the one used for the indolizine compounds, and the potency and ADMET profile proved to be similar.

Transient receptor potential vanilloid 1 (TRPV1)[1] is a non-selective ligand-gated cation channel which upon activation by a range of stimuli such as heat,[2] acid,[3] voltage,[4] exogenous capsaicin[5] and inflammatory mediators such as anandamine[6] and lipoxygenase products[7] allows the flow of sodium and calcium ions through the nerve cell. The increased intracellular Ca^{2+} level causes an excitation of the primary sensory neurons and ultimately leads to the central perception of pain. A TRPV1 knockout mice model provided evidence that thermal hyperalgesia depends on TRPV1 activation and sensitization, making this receptor a very attractive target for the treatment of chronic and neuropathic pain.[8–11]

The pharmaceutical industry responded by undertaking significant efforts in finding small molecule TRPV1 antagonist acting as analgesics and several compounds entered clinical trials.[12–15] Further successful development of these compounds was unfortunately hindered by diverse adverse effects.[12–19] Perhaps the most troublesome is the indication that the TRPV1 channel blockade can lead to hyperthermia as well as an impaired noxious heat perception; in other words that this ion channel plays a role in the body temperature regulation and in setting the heat pain threshold.[20,21] It is however important to put these findings into perspective and to realize that the data available as of today is still limited. Meanwhile, the search for a potent small molecule TRPV1 antagonist with the adequate ADMET (absorption, distribution, metabolism, excretion and toxicity) profile continues.[22]

Previous effort in identifying TRPV1 antagonists relied on the traditional Ca^{2+} influx induced by capsaicin assay and led to the identification of several small molecule TRPV1 antagonists with the progression of few of them to clinical trial. Unfortunately, as aforementioned the presence of side effects put a stop to the further development of many of these compounds.

We hypothesized that these adverse effects could be caused by the inherent properties of the molecule and/or by the TRPV1 activation mode profile. In order to identify new hit compounds having a different phenotypic profile we proceeded with a new HTS campaign wherein the Ca^{2+} influx assay was replaced by a Rb^{+} atomic absorption spectroscopy assay.[23] Because one of the aims was to reduce the lipophilicity of the new hit relative to the clinical

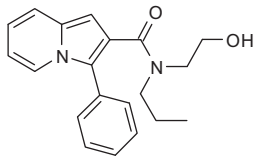

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Figure 1. Novel indolizine TRPV1 antagonist.

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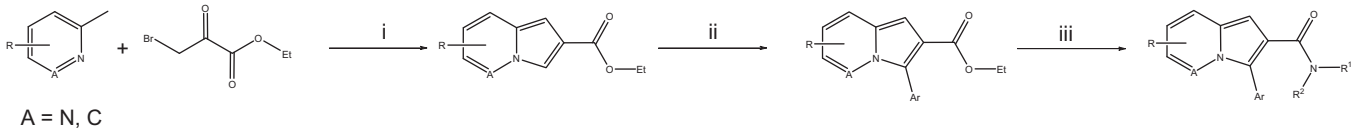
candidates, we placed a special emphasis on the ligand lipophilic efficiency (LLE) parameter with the expectation to obtain a better promiscuity profile.

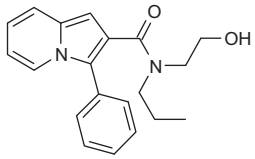
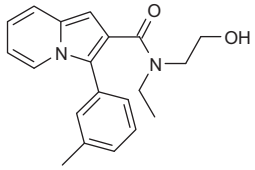
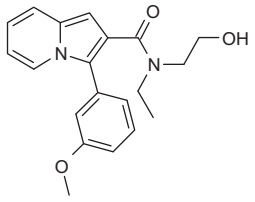
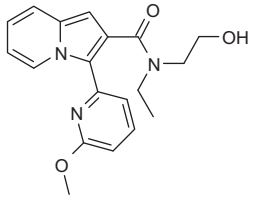
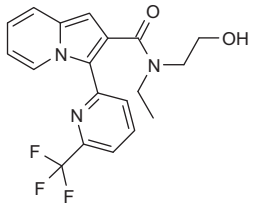
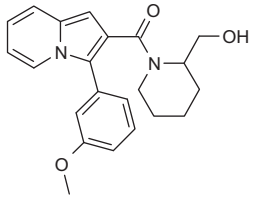
Screening our corporate library led to the identification of indolizine **1** (Fig. 1). This starting point was viewed as attractive because the structure of compound **1** shared few common features with the reported TRPV1 antagonists and has relatively good physical properties (molecular weight 322, *e*Log*D* 3.3). The activity of the hit was subsequently confirmed in a more physiologically relevant assay where an IonWorks™ Quattro (QT) apparatus was used to determine the inhibitory effects of the compound in a human-TRPV1 cell assay as an 8-points concentration–response-curve.[24]

The structure of **1**, an indolizine flanked with a carboxamide and an aryl moiety respectively at the C-6 and C-7 position, provided an opportunity for efficient synthesis of analogues. We have



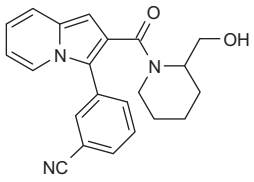
Scheme 1. Reagents and conditions: (i) $NaHCO_3$, ethanol, 120 °C; (ii) ArX, bis(triphenylphosphine)palladium(II)chloride, KOAc, dioxane, 90–110 °C; (iii) aminoalcohol, bis(trimethylaluminum)-1,4diazabicyclo[2.2.2]octane adduct, THF, 40 °C.

Table 1
Early SAR of the indolizine series

Compound	Structure	MW	*e*Log*D* [a]	TRPV1 [b] pIC_{50}	LLE [c]	Solubility [d] (μM)	hERG [e] pIC_{50}	Caco-2 [f] Papp (10^{-6} cm/s)	RLM/HLM [g] Clint (μL/min/mg)	Rat/human GSH trapping [h] (%)
1		322.4	3.3	5.7	2.4	280	ND	60	>500/220	30/NV
2		322.4	3.2	6.8	3.6	260	ND	ND	170/89	ND/ND
3		338.4	2.8	6.0	3.2	150	<4.48	54	65/54	15/31
4		375.4	2.5	5.9	3.4	190	<4.48	56	31/31	25/22
5		377.3	3.6	6.3	2.7	360	ND	ND	48/62	ND/ND
6		364.4	3.3	6.3	3.0	260	<4.48	58	93/160	9/15

(*continued on next page*)

Table 1 (*continued*)

Compound	Structure	MW	*e*Log*D* [a]	TRPV1 [b] pIC_{50}	LLE [c]	Solubility [d] (μM)	hERG [e] pIC_{50}	Caco-2 [f] Papp (10^{-6} cm/s)	RLM/HLM [g] Clint (μL/min/mg)	Rat/human GSH trapping [h] (%)
7		359.4	2.5	5.4	2.9	250	4.61	ND	79/77	51/34

[a] Determined by reversed phase liquid chromatography.
[b] Inhibitory effect against the human transient receptor potential vanilloid 1 (hTRPV1) ligand-gated ion channel expressed in CHO cells measured with an IonWorks™ Quattro (QT) apparatus.
[c] LLE calculated as pIC_{50}–*e*Log*D*.
[d] Dried DMSO solubility, Ref. 25.
[e] Measured in hERG-expressing CHO cells using an IonWorks assay setting, Ref. 26.
[f] Measured permeability (A–B) through Caco-2 cells.
[g] Rat and human liver microsome intrinsic apparent clearance. It is likely that the Clint values are a combination of intrinsic clearance and light chemical decomposition.
[h] Peaks of glutathione adducts are integrated (in the total ion current trace) and the area is divided with the integrated area of the major glutathione adduct of clozapine. The value is reported as GSH conjugate ratio*100, Ref. 27; ND: Not determined; NV: No value.

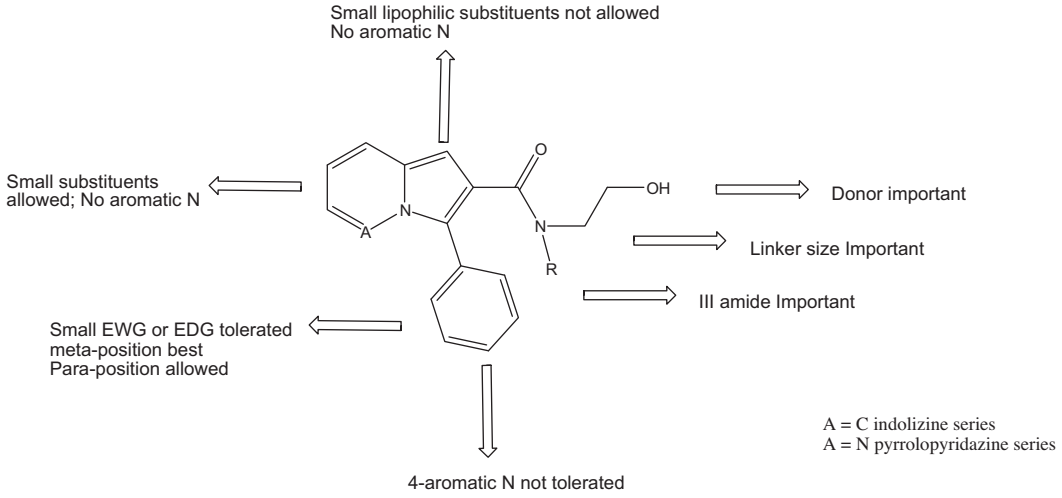
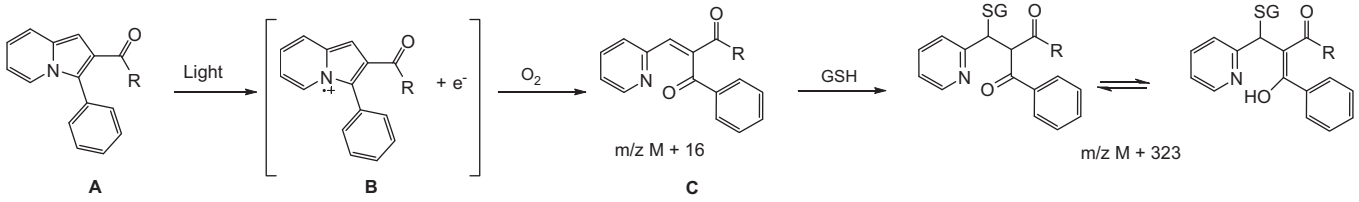


Figure 2. SAR analysis of the indolizine and pyrrolopyridazine series.

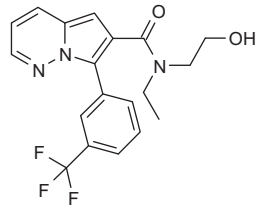
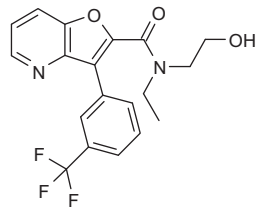
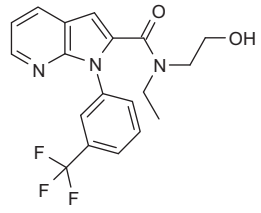
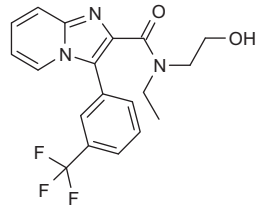
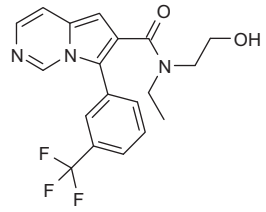
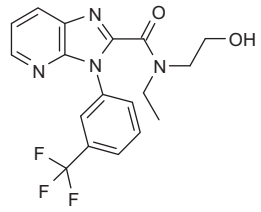


Scheme 2. Putative mechanism for the light and oxygen promoted addition of GSH to the indolizine scaffold.

developed the synthetic route outlined in Scheme 1 that allowed us to quickly evaluate the structure–activity relationship and the ADMET profile within the series. Starting from 2-methylpyridine and ethyl bromopyruvate the indolizine core was first prepared. Then using synthetic conditions that utilize readily available reagent classes (Heck and amide couplings) a number of analogues were readily synthesized (see examples in Table 1). Noteworthy, the sequence order between the Heck reaction and the amide formation could be interchanged.

A selection of the analogues synthesized is outlined in Table 1 as well as some of their associated ADMET data. The SAR will be discussed at a later point (vide infra, see Fig. 2). At this stage of the program, the activity and LLE were judged to be in a decent range (pIC_{50} above 5.4 and LLE above 3; up to 6.8 for **2** with an LLE of 3.6), and while the solubility and the permeability data proved to be in a reasonable range, the clearance was less than optimal. More importantly all the tested compounds showed a propensity to react with glutathione (GSH) when they were incubated with human liver microsome and GSH (see Table 1 GSH trapping). This phenomenon was found to be dependent on the presence of light and oxygen. A mass spectroscopy study, where GSH was added to the compound in the presence of light and oxygen, led us to rationalize the GSH addition could occur as shown in Scheme 2. A single electron transfer oxidation would lead to the reactive

Table 2
Evaluation of diverse scaffolds using the indolizine core as foundation

Compound	Structure	MW	*e*Log*D*[a]	TRPV1[b] pIC_{50}	LLE[c]	Solubility[d] (μM)	RLM/HLM[e] Clint (μL/min/mg)
8		377.4	3.2	7.0	3.8	200	130/91
9		378.4	3.2	6.3	3.1	>400	13/43
10		377.4	2.8	6.0	3.2	>300	<10/32
11		377.4	2.4	NV	NV	270	<10/14
12		377.4	2.0	NV	NV	240	29/30
13		378.4	3.8	NV	NV	24	<10/<10

[a] Determined by reversed phase liquid chromatography.
[b] Inhibitory effect against the human transient receptor potential vanilloid 1 (hTRPV1) ligand-gated ion channel expressed in CHO cells measured with an IonWorks™ Quattro (QT) apparatus.
[c] LLE calculated as pIC_{50}–*e*Log*D*.
[d] Dried DMSO solubility, Ref. 25.
[e] Rat and human liver microsome intrinsic apparent clearance; NV: No value.

intermediate **B** which in the presence of oxygen would give rise to the Michael acceptor **C**, which in turn would readily react with GSH to form the M+323 adduct detected in the LCMS experiment. When tested against TRPV1 compound **C** was shown to lose all activity.

Faced with this light stability issue, we then embarked on the search of a more stable scaffold that uses the indolizine core as the foundation. We hypothesized that the addition of a heteroatom onto the bicyclic core structure would serve two purposes, improving the light stability by modifying the electron density of the ring system and improving the metabolic stability by reducing the lipophilic character of the compound which was also an issue associated with the indolizine series. In order to check this hypothesis a selected set of compounds was synthesized.[28] The associated set of data is outlined in Table 2. Only few new scaffolds proved to retain

Table 3
SAR of the pyrrolopyridazine series

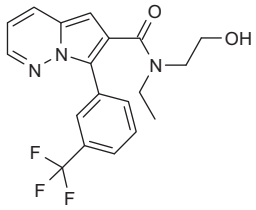
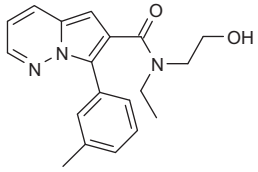
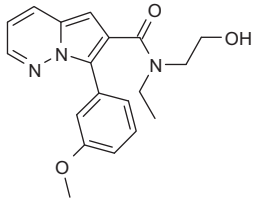
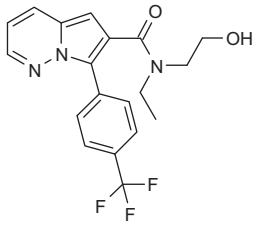
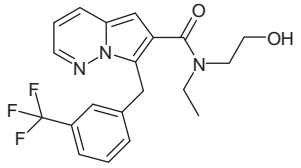
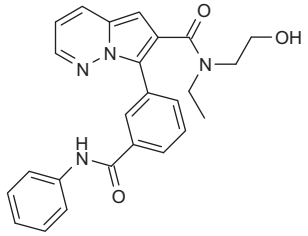
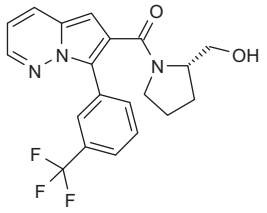
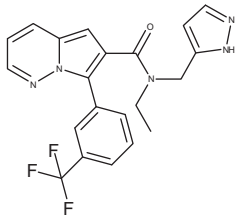
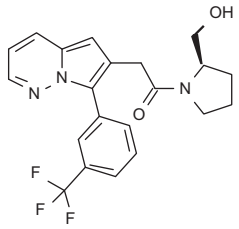
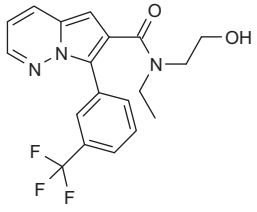
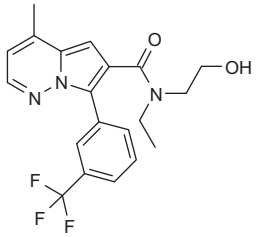
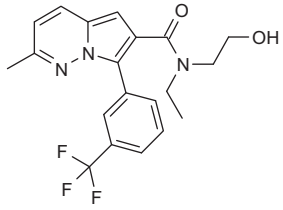
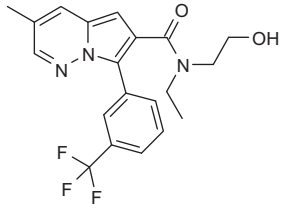
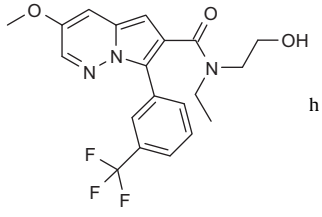
Compound	Structure	MW	*e*Log*D*[a]	TRPV1[b] pIC_{50}	LLE[c]	Solubility[d] (μM)	hERG[e] pIC_{50}	Caco-2[f] Papp (10^{-6} cm/s)	RLM/HLM[g] Clint (μL/min/mg)
8		377.4	3.2	7.0	3.8	200	4.63	42	130/91
14		323.4	2.3	6.6	4.3	330	<4.5	ND	130/23
15		339.4	1.9	5.9	4.0	340	ND	ND	130/19
16		377.4	3.3	5.6	2.3	22	ND	ND	47/78
17		391.4	3.8	5.1	1.3	170	ND	ND	>500/>500
18		428.5	2.4	NV	NV	25	ND	ND	75/55
19		389.4	3.7	6.7	3.0	360	ND	ND	100/99
20		413.4	3.5	NV	NV	150	ND	ND	500/360

Table 3 (*continued*)

Compound	Structure	MW	*e*Log*D*[a]	TRPV1[b] pIC_{50}	LLE[c]	Solubility[d] (μM)	hERG[e] pIC_{50}	Caco-2[f] Papp (10^{-6} cm/s)	RLM/HLM[g] Clint (μL/min/mg)
21		403.4	3.7	5.8	2.1	220	ND	ND	140/150

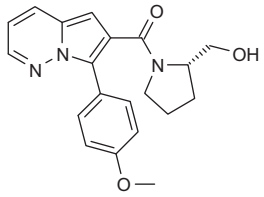
[a] Determined by reversed phase liquid chromatography.
[b] Inhibitory effect against the human transient receptor potential vanilloid 1 (hTRPV1) ligand-gated ion channel expressed in CHO cells measured with an IonWorks™ Quattro (QT) apparatus.
[c] LLE calculated as pIC_{50}–*e*Log*D*.
[d] Dried DMSO solubility, Ref. 25.
[e] Measured in hERG-expressing CHO cells using an IonWorks assay setting, Ref. 26.
[f] Measured permeability (A–B) through Caco-2 cells.
[g] Rat and human liver microsome intrinsic apparent clearance; ND: Not determined; NV: No value.

Table 4
Attempts to improve metabolic stability

Compound	Structure	MW	*e*Log*D*[a]	TRPV1[b] pIC_{50}	LLE[c]	Solubility[d] (μM)	RLM/HLM[e] Clint(μL/min/mg)
8		377.4	3.2	7.0	3.8	200	130/91
22		391.4	3.7	6.2	2.5	32	84/87
23		391.4	3.8	6.1	2.3	28	130/170
24		391.4	3.6	5.3	1.7	190	320/120
25	 h	407.4	3.7	5.3	1.6	25	110/91

(*continued on next page*)

Table 4 (*continued*)

Compound	Structure	MW	*e*Log*D*[a]	TRPV1[b] pIC_{50}	LLE[c]	Solubility[d] (μM)	RLM/HLM[e] Clint(μL/min/mg)
26		351.4	2.2	5.5	3.3	>400	33/10

[a] Determined by reversed phase liquid chromatography.
[b] Inhibitory effect against the human transient receptor potential vanilloid 1 (hTRPV1) ligand-gated ion channel expressed in CHO cells measured with an IonWorks™ Quattro (QT) apparatus.
[c] LLE calculated as pIC_{50}–*e*Log*D*.
[d] Dried DMSO solubility, Ref. 25.
[e] Rat and human liver microsome intrinsic apparent clearance.

the indolizine hTRPV1 activity and while the variation of the TRPV1 activity across the set of compounds could be attributed to a specific interaction between the bicyclic core and the target, a rational overall structure relationship activity remains elusive. The light sensitivity of the relevant scaffolds (**8**–**10**) was then assessed and these compounds proved to be inert to UV-light (254 nM) exposure when tested as a 1 mM DMSO solution. Under the same conditions the initial indolizine scaffold was shown to be unstable and was fully converted to the Michael acceptor **C** (Scheme 2).

Among these compounds (see Table 2), pyrrolopyridazine **8** exhibited good potency, a relatively good ADME profile and an increased LLE as compared to the initial indolizine scaffold, in other words the best overall profile (all data not shown). Encouraged by these data, we then set out to evaluate the potential of this new series. The synthesis of the pyrrolopyridazine compounds follows the same steps as the indolizine series (see Scheme 1). In general, the yields of the cyclization reaction proved to be lower, which could be attributed to the weaker nucleophilic character of the pyridazine ring.

A first iteration of compounds indicated that the early SAR established for the indolizine compounds correlated well with the pyrrolopyridazine SAR. Guided by the SAR analysis of the indolizine series, a subsequent iteration of synthesis led to a quick evaluation of the SAR and ADME properties for this series (Table 3). Substitution at the meta-position of the phenyl moiety such as in **8** led to the most beneficial effect, while substitution at para-position such as in **16** was tolerated, albeit with a potency drop. Small lipophilic substituents at the meta-position of the phenyl group, such as a methyl (**14**) or a trifluoromethyl group (**8**) proved to be the most beneficial for the potency and the LLE; more hydrophilic substituent such as the methoxy group in **15** was tolerated but was accompanied with a substantial drop in potency. Cumulating two small substituents did not lead to any beneficial effect (data not shown). The replacement of the phenyl ring with a benzylic moiety (**17**) or the extension of the phenyl ring with a phenylcarboxamide moiety (**18**) was detrimental to the activity.

On the amide side, the H-bond donor group proved to be crucial for the potency as well as the two carbons linker between the amide and the H-bond donor atom. Only tertiary amides showed activity while secondary amide exhibited no activity (data not shown). The fact that the size of the linker between the hydroxyl group and the amide moiety and that only tertiary amides were active led us to hypothesize the formation of a hydrogen bond between the hydroxyl moiety and the amide heteroatoms placing the compound into a more favorable conformation (closer to the bioactive conformation). To quickly evaluate this hypothesis, cyclopentyl **19** was synthesized but while it demonstrated similar level of activity as compare to **8**, the anticipated boost of potency was not achieved. Noteworthy the other enantiomer was not active. The integration of the H-bond donor group onto an aromatic ring such as pyrazole **20** was also plagued with a total loss of activity. Introduction of a carbon atom spacer between the pyrrolopyridazine and the amide in **21** was also received with very little success (the enantiomer showed no activity). A summary of the SAR findings is depicted in Figure 2.

The overall profile of this series was relatively good as indicated in Table 3. However as in the case of the indolizine series the metabolic stability proved to be less than optimal for the pyrrolopyridazine series. We investigated the use of small substituents on the pyridazine ring such as the methyl in compounds **22–24** and the methoxy group in **25**, hypothesizing that the pyridazine ring could be the primary site of metabolism (see Table 4). However none of the compounds showed any improved metabolic stability and even worse, the potency and the LLE was lower.

Realizing that the lipophilic character of the compound could play an important role in the metabolic stability of the series, we focus our attention to the small subset of compounds having an *e*Log*D* < 3. For most compounds, keeping an *e*Log*D* < 3 proved to be unfruitful for improving the rat microsome stability, however, the human microsome stability was impacted positively (see for example compounds **14**, **15** and **18**, Table 3). Looking back into the clearance activity relationship of the indolizine series (Table 1), we noticed that a more rigid/bulky moiety alpha to the hydroxyl group, such as the cyclohexyl ring in compounds **6** and **7**, displayed a positive effect on the rat clearance data. Gratifyingly, by combining both factors, *e*Log*D* kept below 3 and a less flexible hydroxylamide moiety such as in compound **26** (Table 4), a substantial metabolic stability improvement was achieved.

In summary, a new class of TRPV1 antagonist was identified from an HTS campaign that used an Rb^+ atomic absorption read out. This indolizine class of compounds proved to be unstable in the presence of light and oxygen and we have demonstrated that the pyrrolopyridazine scaffold can be used to address this issue. This class of compounds exhibited the same level of potency, no light stability issue and a similar DMPK profile. The metabolic stability liability inherent to the indolizine class remained a problem for the pyrrolopyridazine scaffold. However, the appropriate selection of small substituents on the aromatic pendant (keeping the *e*Log*D* below 3) combined with the use of a more bulky amide alleviated this issue and still provided potent TRPV1 antagonists.

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