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Synthesis and Evaluation of Isatins and Thiosemicarbazone Derivatives against Cruzain, Falcipain-2 and Rhodesain

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Abstract—While commercial isatins were practically inactive against the target proteases, thiosemicarbazone derivatives were found to be active. The most active compound from the series displayed an inhibitory IC_{50} value of 1 μM against rhodesain. One thiosemicarbazone was found to be active against all three proteases with inhibitory IC_{50} values of 10 μM or less. A combination of *N*-benzylation and appropriate substitution on the aromatic portion of the isatin scaffold was generally found to be beneficial especially against cruzain for ketone inhibitors.

Isatin **1** is a natural product found in a number of plants including those of the genus *Isatis*.[1] It has also been found as a metabolic derivative of adrenaline in humans.[2] Various derivatives of isatin are known to possess a range of pharmacological properties including antiprotozoal activities.[3,4] Within the context of enzyme inhibitors, isatins (also known as 2,3-dioxindoles) have seen recent applications in the inhibition of cysteine and serine proteases.[5,6] Thus isatin is a biologically validated starting point for the design and synthesis of chemical libraries directed at these targets.[7] Due to the privileged nature of isatin, libraries designed and synthesized around the basic structure of this scaffold should yield medicinally active compounds with high hit rates at significantly reduced library size compared to large classical libraries obtained from combinatorial chemistry efforts based on non-privileged templates.

The aforementioned antiprotozoal and cysteine protease inhibitory activities of isatin derivatives prompted us to investigate this class of compounds as potential ketone inhibitors of parasitic cysteine proteases identified in trypanosomes (cruzain and rhodesain) and malaria parasites (falcipain-2) respectively.[8–11] It is noteworthy that a series of highly potent reversible ketone inhibitors of cruzain have previously been disclosed.[12] This has recently culminated in the first crystal structures of these inhibitors being solved.[13] We reasoned that the presence of the ketone functionality adjacent to the aromatic ring on the isatin scaffold provides a diversity point for accessing the corresponding thiosemicarbazones, a class of compounds recently identified as potent anti-trypanosomal inhibitors of cruzain.[14] Coupled with the molecular simplicity, potentially cost effective synthesis and non-peptidic nature, this makes isatins attractive scaffolds for antiparasitic drug discovery. Cost-effective synthesis should always be a consideration in deciding on potential new antiparasitic agents given the prevalence of major parasitic diseases in poor countries.

The initial aim of our work on isatins is focused on identifying the most promising commercially available scaffold and ideal sites for introducing chemical diversity before launching a full scale medicinal chemistry programme. In this paper, we report on the evaluation of isatin-based synthetic thiosemicarbazones **2** and *N*-substituted isatins **3** against cruzain, falcipain-2 and rhodesain (Fig. 1).

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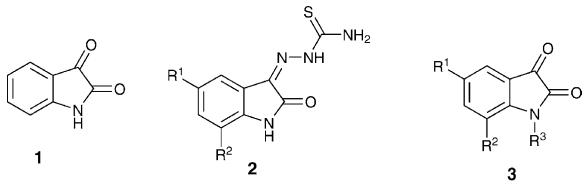
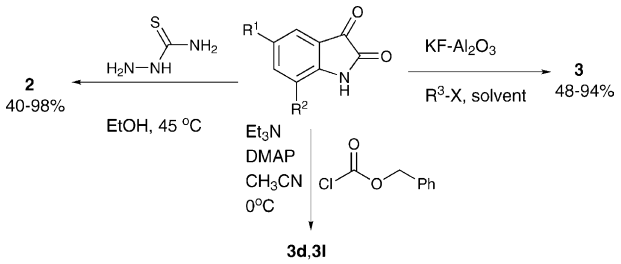


Figure 1.

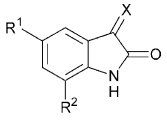


Scheme 1.

Scheme 1 depicts the straightforward and simple synthesis of target compounds from commercially available isatins. The simplicity of the chemistry cannot be overemphasised. Reaction of commercially available isatins with thiosemicarbazide in ethanol provided target thiosemicarbazones **2**. Isatins **3** were synthesized via alkylation, acylation and sulfonylation of the same commercially available starting materials. Acetonitrile was used as the solvent for all alkylation reactions while tetrahydrofuran was used for all acylation and sulfonylation reactions. The reactions mediated by potassium fluoride supported on alumina provided derivatives in generally high yields and purity as determined by ^{1}H NMR analysis before further purification by recrystallisation to obtain analytically pure samples. The results obtained with this support-bound base augur well for the high throughput parallel solution phase synthesis of libraries of these derivatives when used in conjunction with scavenging protocols. Alkylation reactions of isatins using a support-bound base, 2-*tert*-butylimino-2-diethylamino-1,3-dimethylperhydro-1,3,2-diazaphosphorine on polystyrene (BEMP) in conjunction with scavenging protocols have been reported.[7] The major drawback of BEMP in these reactions is the very high cost of this base. On the other hand KF-Al_2O_3 is relatively inexpensive and easy to prepare. The use of this support-bound base in a variety of reactions is the subject of a recent review.[15] Its envisaged use in the synthesis of *N*-alkylated thiosemicarbazones compliments a recent literature report on the solid phase synthesis of isatin β-thiosemicarbazone Mannich bases. In this report a trityl resin-supported thiosemicarbazide captures isatins from solution, permits *N*-alkylation and is then easily removed from the solid support.[16] Carbamates **3d** and **3e** were prepared from isatins using benzylchloroformate in the presence of triethylamine and 4-dimethylaminopyridine. All new compounds gave ^{1}H NMR, FAB-MS and microanalysis data consistent with their structures.

IC_{50} values against recombinant proteins (cruzain and falcipain-2) were determined essentially as described previously.[14,17] IC_{50} values for rhodesain (recombinant, cysteine protease from *T. brucei rhodensiense*, EC 3.4.22.X) were similarly determined at 3 nM. The results are presented in Tables 1 and 2. Data for commercially available isatins is included for comparison purposes. From Table 1, it is immediately clear that commercially available isatins were practically inactive while the corresponding thiosemicarbazones showed some activity (albeit modest in many cases). The most notable

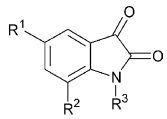
Table 1. Inhibition of cruzain, falcipain-2 and rhodesain by commercial isatins and thiosemicarbazone derivatives **2**

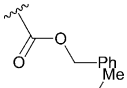
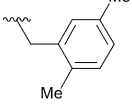
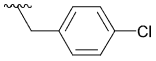
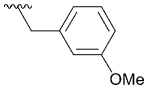
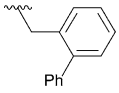
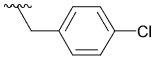
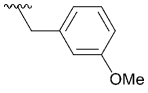
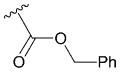
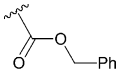


Compd	R^1	R^2	X	IC_{50} (μM)		
				Cruzain	Falcipain-2	Rhodesain
Isatin	H	H	O	> > 10	NE	> > 10
5-Methylisatin	Me	H	O	> > 10	NE	ND
5-Fluoroisatin	F	H	O	> > 10	NE	ND
5-Chloroisatin	Cl	H	O	> > 10	NE	ND
5-Bromoisatin	Br	H	O	> > 10	NE	ND
5-Iodoisatin	I	H	O	> > 10	NE	ND
5-Nitroisatin	NO_2	H	O	> > 10	42.6	> > 10
5-(Trifluoromethoxy)isatin	CF_3O	H	O	> > 10	NE	> > 10
5,7-Dimethylisatin	Me	Me	O	> > 10	NE	ND
2a	H	H	N–NHC(S)NH_2	8	38.8	3.5
2b	Me	H	N–NHC(S)NH_2	20–50	43.9	15
2c	F	H	N–NHC(S)NH_2	30	38.6	15
2d	Cl	H	N–NHC(S)NH_2	21	32.8	6
2e	Br	H	N–NHC(S)NH_2	20	29.6	7
2f	I	H	N–NHC(S)NH_2	9	28.7	1
2g	NO_2	H	N–NHC(S)NH_2	30	4.4	17
2h	Me	Me	N–NHC(S)NH_2	16	13.2	15
2i	Cl	Me	N–NHC(S)NH_2	10.5	9.4	3

NE, no effect; ND, not determined.

Table 2. Inhibition of cruzain, falcipain-2 and rhodesain by *N*-substituted isatins derivatives **3**



Compd	R^1	R^2	R^3	IC_{50} (μM)		
				Cruzain	Falcipain-2	Rhodesain
1-Methylisatin	H	H	Me	> > 10	NE	ND
1-Phenylisatin	H	H	Ph	> > 10	NE	ND
3a	H	H		> > 10	NE	> > 10
3b	H	H		> > 10	NE	15
3c	H	H		> > 10	NE	100
3d	H	H		> > 10	NE	30
3e	Me	H		80	NE	> > 10
3f	Me	H		> > 10	NE	> > 10
3g	Me	H		2	21.9	> > 10
3h	Me	H		2.8	9.2	> > 10
3i	Cl	H		6	31.2	> > 10
3j	Cl	H		> > 10	34.6	> > 10
3k	Cl	H		90	46.9	> > 10
3l	Cl	H		80	NE	> > 10
3m	I	H		90	ND	> > 10
3n	F	H		> > 10	ND	> > 10

NE, no effect; ND, not determined.

thiosemicarbazone scaffold from this limited series is **2i** which showed activity against all targets with IC_{50} values of 10 μM or less. The most promising scaffolds in the series for the homologous cruzain and rhodesain are **2a** and **2f** while, in addition to **2i**, thiosemicarbazone **2g** provides a potential scaffold for future development of falcipain-2 inhibitors.

Although the number of compounds synthesized is small, from the data presented in Table 2, it is clear that lack of substitution at the 5-position (R^1 substituent) of isatin did not generally improve activity. Results from the commercially available 1-methylisatin and 1-phenylisatin as well as from compounds **3a–3d** confirm this. In comparison to the data for *N*-unsubstituted commercially

available isatins (Table 1), it is evident that *N*-benzylation in combination with substitution at position 5 of the isatin scaffold seems favorable. Cruzain was the most sensitive protease to *N*-benzylation of the isatin scaffold with rhodesain being the least sensitive.

The most notable compound from this limited series of *N*-substituted derivatives is **3h** which shows activity against both cruzain ($IC_{50} = 2.8\,\mu M$) and falcipain-2 ($IC_{50} = 9.2\,\mu M$). It is noteworthy that this compound contains a privileged biphenyl substructural motif.[18] The increased potency against cruzain of compounds arising from *N*-benzylation may be attributed to the ability of the benzyl moiety to bind more tightly in the S_2 pocket of the active site of the enzyme.

In conclusion, we have demonstrated the potential of isatin derivatives to inhibit parasitic cysteine proteases. Based on the preliminary data presented in Tables 1 and 2, and in terms of further development and structure–activity studies, combinations of the thiosemicarbazone moiety with substitution at position 5 (R^1 substituent) of the isatin scaffold as well as *N*-benzylation with a broad range of biaryl-containing substituted benzyl electrophiles is certainly worth pursuing. The commercial availability of structurally diverse boronic acids which can be coupled via Suzuki cross coupling chemistry to the *ortho*, *meta*, and *para* positions of bromo-substituted benzyl bromides opens up many interesting possibilities. Work in this regard along with extended structure–activity relationship studies and data against cultured parasites will be reported in a future full paper.

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