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# Acylideneoxoindoles: A new class of reversible inhibitors of human transglutaminase 2

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ABSTRACT

Inhibitors of human transglutaminase 2 (TG2) are anticipated to be useful in the therapy of a variety of diseases including celiac sprue as well as certain CNS disorders and cancers. A class of 3-acylidene-2-oxoindoles was identified as potent reversible inhibitors of human TG2. Structure–activity relationship analysis of a lead compound led to the generation of several potent, competitive inhibitors. Analogs with significant non-competitive character were also identified, suggesting that the compounds bind at one or more allosteric regulatory sites on this multidomain enzyme. The most active compounds had $K_i$ values below 1.0 μM in two different kinetic assays for human TG2, and may therefore be suitable for investigations into the role of TG2 in physiology and disease in animals.



Transglutaminase 2 (TG2), a ubiquitous member of the mammalian transglutaminase enzyme family, is found in intracellular as well as extracellular environments of many organs. In the presence of $Ca^{2+}$ and the absence of guanine nucleotides, TG2 adopts an open, catalytically competent conformation, which activates γ-glutaminyl residues on proteins as acyl donors and cross-links these to ε-amino groups of lysyl residues. As a result, proteolytically resistant isopeptide bonds are formed between proteins. Hydrolysis of the γ-glutamylacyl-enzyme intermediate results in deamidation of the substrate.[1,2] TG2 is implicated in the pathogenesis of disorders including neurological diseases such as Huntington's, Alzheimer's and Parkinson's diseases, certain types of cancers and renal diseases, cystic fibrosis and celiac sprue,[3–8] and may therefore be a suitable therapeutic target for one or more of these conditions.[9] Consequently, small molecule modulators of in vivo TG2 activity are of pharmacological and medicinal interest.

Several classes of irreversible inhibitors of TG2 have been described thus far (Fig. 1).[2,10–15] More recently, three classes of reversible inhibitors have also been reported.[16–18] Here, we present a structure–activity relationship (SAR) analysis for a new class of reversible inhibitors of human TG2, the acylideneoxoindoles.

Isatin (indoline-2,3-dione) is an endogenous indole in mammals with a range of biological activities.[19,20] Our motivation to screen this natural product as a candidate TG2 inhibitor was guided by the hypothesis that the cyclic α-keto amide structure of isatin may mimic the γ-carboxamide group of TG2 substrates. α-Keto amides, including isatin analogs, are widely utilized as reversible inhibitors of cysteine-dependent proteases.[21] This led us to propose that isatin analogs may also be reversible inhibitors of the cysteine transglutaminase TG2. In preliminary screening efforts, isatin was found to be a weak, reversible inhibitor of human TG2 ($IC_{50}$ >0.25 mM), and certain five-substituted analogs with electron-withdrawing functional groups were somewhat more active ($IC_{50}$ = 65–450 μM for 5-chloro, 5-bromo, 5-iodo, and 5,7-difluoroisatin).

Using this information and data available for other classes of TG2 inhibitors, we built a ligand-based statistical model with which to identify new TG2 inhibitors. This model was used to screen ChemNavigator's iResearch library of commercially available compounds, and to prioritize compounds for acquisition and testing. Among these were a series of symmetrical isatin dimers (**1**–**6**), as well as three 3-acylidene-2-oxoindoles: indirubin (**7**), isoindigotin (**8**), and methyl ketone (**9**) (Table 1).

Using a standard glutamate dehydrogenase (GDH)-coupled deamidation assay with Cbz-Gln-Gly (ZQG) as the acyl donor substrate,[22] isatin dimers linked 6,6′ (**1**), 5,5′ (**2**, **3**), and 1,1′ (**4**, **5**) were

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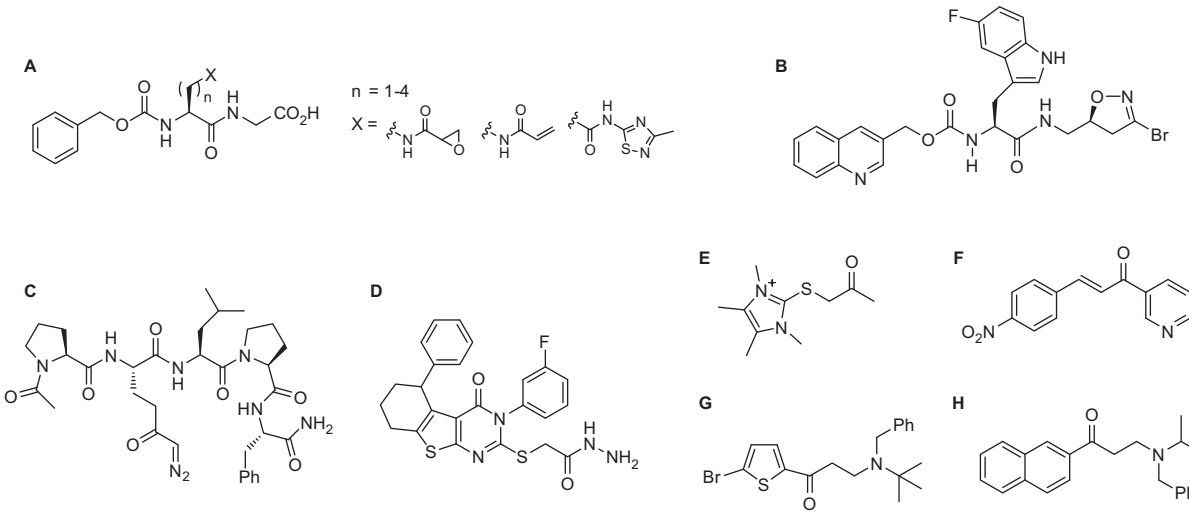


**Figure 1.** Selected TG2 inhibitors—irreversible dipeptide inhibitors (A),[11] irreversible DHI-based inhibitors (B),[10] irreversible DON-based substrate mimics (C),[2] reversible thienopyrimidinones (D),[16] irreversible imidazolium salts (E),[12,13] reversible azachalcones (F)[17] and aryl-β-aminoethylketones (G and H).[14,15]

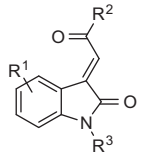


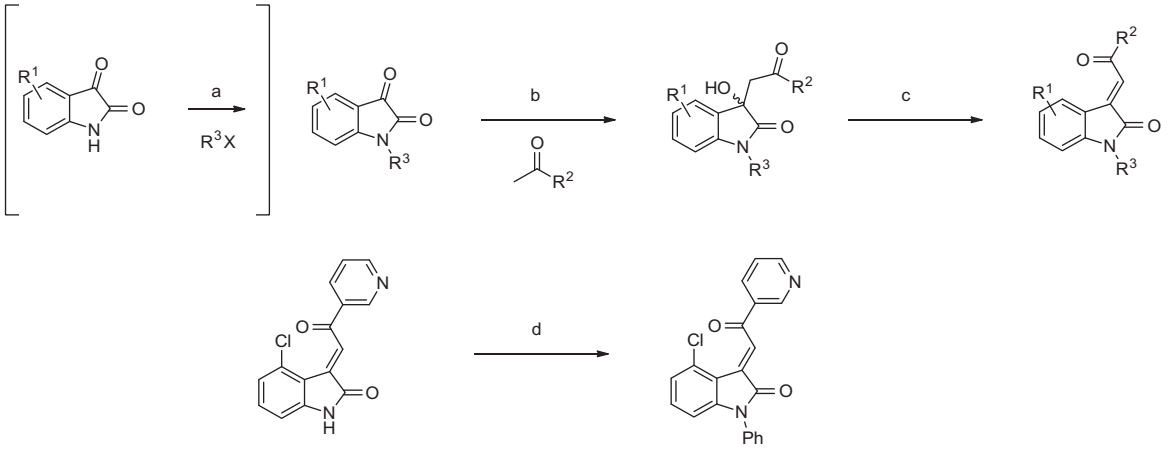
**Figure 2.** Acylidene oxoindoles.

found to display inhibition constants in the range of 18–40 μM, approximately 10-fold more potent than the simple 5-haloisatins. The linker can play a role in determining the activity of isatin dimers: the *m*-xylyl and methylene-linked analogs **4** and **6** were active whereas the *p*-xylyl linked analog **5**, a constitutional isomer of **4**, was not. Among the 3-acylidene oxoindoles, indirubin (**7**) was inactive, but isoindigotin (**8**) and the *E*-methyl ketone **9** proved to be promising inhibitors.

To explore the potential of acylidene oxoindoles as TG2 inhibitors, we undertook the synthesis of analogs of compound **9** bearing substitution in three regions—on the aromatic oxoindole ring ($R^1$), at the methyl position of the ketone ($R^2$), and on the amide nitrogen ($R^3$) (Fig. 2).

The acylidene oxoindoles were prepared by a two-step condensation–dehydration sequence from isatin or a substituted isatin along with acetone or an aryl methyl ketone (Scheme 1). The first step, performed under basic conditions, afforded β-hydroxy ketones which were isolated and then dehydrated under acidic conditions, or via the agency of methane sulfonyl chloride in pyridine, to produce the acylidene oxoindole.[23] All compounds were obtained as a single stereoisomer, which was assigned as the (*E*)-diastereomer based on the $^1$H NMR spectra, which displayed downfield chemical shifts for the aromatic C-4 proton resonances.[24,25] N-Substituted compounds were prepared either via condensation–dehydration starting from the corresponding N-substituted isatin or via copper-mediated N-arylation of an acylidene oxoindole.[26]

The inhibitory properties of the acylidene oxoindoles toward TG2-catalyzed deamidation of ZQG were initially characterized using the GDH-coupled assay (Table 2). We first examined a small series of analogs of parent compound **9** bearing fluoro, chloro or ether substituents at the 4-, 5-, 6- or 7-position of the oxoindole system (compounds **10**–**14**). Here, the 4-chloro analog **10** exhibited the highest potency, with an $IC_{50}$ value of 1.5 μM and a $K_i$ value of 0.7 μM.



**Scheme 1.** Synthesis of 3-acylidene-2-oxoindoles. Top: synthesis of N1-H or N1-substituted analogs via condensation–dehydration of N1-H or N1-substituted isatins. Bottom: synthesis of N1-substituted analogs via N-arylation of N1-H compounds: Reagents and conditions: (a) $R^3X$ (alkyl bromide or iodide), $K_2CO_3$, DMF, 16–48 h; (b) acetone, $NHEt_2$, 60 °C, 16 h or aryl methyl ketone, $NHEt_2$, EtOH, rt, 2–48 h; (c) HCl, AcOH, reflux, 0.5 h or HCl, AcOH, rt, 16 h; (d) $PhB(OH)_2$, $CuSO_4{\cdot}5H_2O$, pyridine, DCM, 16 h.