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SAR of a New Antischistosomal Urea Carboxylic Acid

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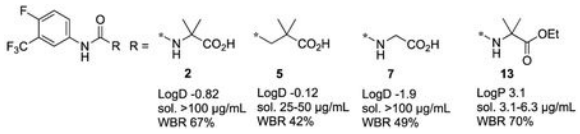
Abstract

Urea carboxylic acids, products of aryl hydantoin hydrolysis, were recently identified as a new antischistosomal chemotype. We now describe a baseline structure-activity relationship (SAR) for this compound series. With one exception, analogs of lead urea carboxylic acid **2** were quite polar with $LogD_{7.4}$ values ranging from −1.9 to 1.8, had high aqueous solubilities in the range of 25-100 μg/mL, and were metabolically stable. None of the compounds had measurable in vitro antischistosomal activity or cytotoxicity, but four of these had moderate worm burden reduction (WBR) values of 42-70% when they were administered as single 100 mg/kg oral doses to *S. mansoni*-infected mice. These data indicate that with the exception of the *gem*-dimethyl substructure and the distal nitrogen atom of the urea functional group, the rest of the structure of **2** is required for in vivo antischistosomal activity.

Graphical Abstract



Keywords

urea carboxylic acid; antischistosomal; SAR

The aryl hydantoin Ro 13-3978 is a promising antischistosomal lead compound (Figure 1) despite its weak antiandrogenic properties.[1-4] Although Ro 13-3978 has little effect on

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cultured adult *S. mansoni*,[3] it has high oral efficacy against *S. mansoni*, *S. haematobium* and *S. japonicum* in a variety of animal models.[2,3] For example, Ro 13-3978 has a single oral dose ED_{50} of 15 mg/kg in the *S. mansoni* mouse model; in this same model, praziquantel has an ED_{50} of 172 mg/kg.[3]

Ro 13-3978 has high metabolic stability,[3,4] but when we administered a 100 mg/kg oral dose of this aryl hydantoin to mice, we observed low levels of hydroxymethyl metabolite **1** and urea carboxylic acid **2**, the hydantoin hydrolysis product[5] (Figure 1). Based on $AUC_{0\text{-}24h}$ values, the respective exposures of **1** and **2** were 3 and <1% relative to that of Ro 13-3978.[4] At the same 100 mg/kg dose, metabolite **1** was inactive, whereas **2** reduced worm burden in *S. mansoni*-infected mice by 67%[4] and thus has some promise as a new antischistosomal chemotype. We now describe physicochemical profiling, in vitro ADME, and in vivo activities against *S. mansoni* for a number of analogs of urea carboxylic acid **2** (**3-13**, Table 1) designed to increase antischistosomal efficacy and establish a baseline structure-activity relationship (SAR) for this chemotype.

Target compounds **4-13** were prepared (Supporting Information) by a variety of reactions described in Schemes 1-5. Urea carboxylic acids **7-9** were prepared in reactions between phenyl isocyanate **14**[6] and amino acids **15-17** in low to moderate yields (Scheme 1). Similarly, urea ester **13** was prepared in a reaction between **14** and amino ester **18** in 64% yield.

Urea amide **12** was prepared from **2** in a two-step reaction. First, **2** was converted to *N*-hydroxysuccimide active ester **19** which was then exposed to cone, ammonium hydroxide to form **12** in 29% overall yield (Scheme 2).

Urea carboxylic acid **4** was prepared in 42% yield by treatment of Ro 13-3978 with sodium hydride followed by 1-bromo-3-chloropropane (Scheme 3). Carboxy amide **5** was prepared by acylation of aniline **20** with the corresponding anhydride in 93% yield whereas carboxy amide **6** was prepared by acylation of *gem*-dimethyl glycine with acid chloride **21** in 44% yield (Scheme 3).

The synthesis of imidazolone carboxylic acid **10** began with conversion of aniline **22**[7] to its isocyanate and subsequent reaction with the *tert*-butyl ester of *gem*-dimethyl glycine to afford urea ester **23** in 63% yield (Scheme 4). Exposure of **23** to CuI and DBU in hot DMSO effected ring closure to **24** in 72% yield. Ester hydrolysis of **24** afforded **10** in 97% yield.

The synthesis of dihydropyrimidinone carboxylic acid **11** began by conversion of benzonitrile **25**[7] to benzaldehyde **26** with DIBAL in 33% yield. Reductive amination to amino ester **27** (53%) followed by ring closure with carbonyldiimidazole afforded **28** (93%). Ester hydrolysis of **28** furnished **11** in 70% yield. Known target compound **3** was prepared according to the method of Durini et al.[8]

We now consider the physicochemical and in vitro ADME properties of these analogs of **2** (Table 1). With the exception of amide **12** and ethyl ester **13**, all compounds were quite polar with calculated $LogD_{7.4}$ values below 0 and high aqueous solubilities in the range of 25-100 μg/mL. In spite of this high polarity, **2** exhibited rapid and extensive absorption following

oral administration to mice at a dose of 100 mg/kg (Figure 2) suggesting that the polarity does not limit oral exposure. Consistent with their high polarity, these compounds were metabolically stable with low intrinsic clearance values (corresponding to in vitro half-lives in excess of 200 min) in human and mouse liver microsomes. The only exception to this was ethyl ester **13** which underwent non-NADPH mediated degradation, presumably by non-specific esterases present in the microsomal test systems.

Similar to **2**,[4] **3-13** had no activity against schistosomula (NTS) or cultured adult *S. mansoni*[9] at a 10 μM concentration (data not shown). In contrast, praziquantel has an LC_{50} of 77 nM against adult *S. mansoni* in vitro.[10] To assess host cell cytotoxicity, target compounds were tested for growth inhibition of the rat myoblast L6 cell line; these cells were unaffected at compound concentrations up to 200 μM (data not shown).

In vivo antischistosomal activity was determined by measuring worm burden reduction (WBR) values following administration of single 100 mg/kg oral doses to *S. mansoni*-infected mice (Table 1). As we had previously observed for **2**,[4] none of the compounds tested had high in vivo activity with WBR values >90%, although **5**, **7**, and **13** had moderate WBR values of 42, 49, and 70%, respectively, the latter being statistically significant ($p=0.006$). These data indicate that with the exception of the *gem*-dimethyl substructure and distal nitrogen atom of the urea functional group, the rest of the structure of **2** is required for antischistosomal activity. Further, **4**, **10** and **11** reveal that conformational restriction is not tolerated. Even though none of these compounds had high in vivo activity, it is instructive to note that at this same 100 mg/kg dose, praziquantel reduces worm burden by only 15%[10]

Drug discovery starting with active metabolites[11,12] can be a fruitful strategy. However, in this initial SAR investigation, we were not able identify an analog of **2** with a superior ADME and antischistosomal profile, although our data does suggest that a prodrug strategy could be useful.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgment

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Urea carboxylic acids, a new antischistosomal chemotype.

Baseline structure-activity relationship (SAR).

Analogs were quite polar, had high aqueous solubility, and were metabolically stable.

Modifications to the *gem*-dimethyl and urea functional group were tolerated.

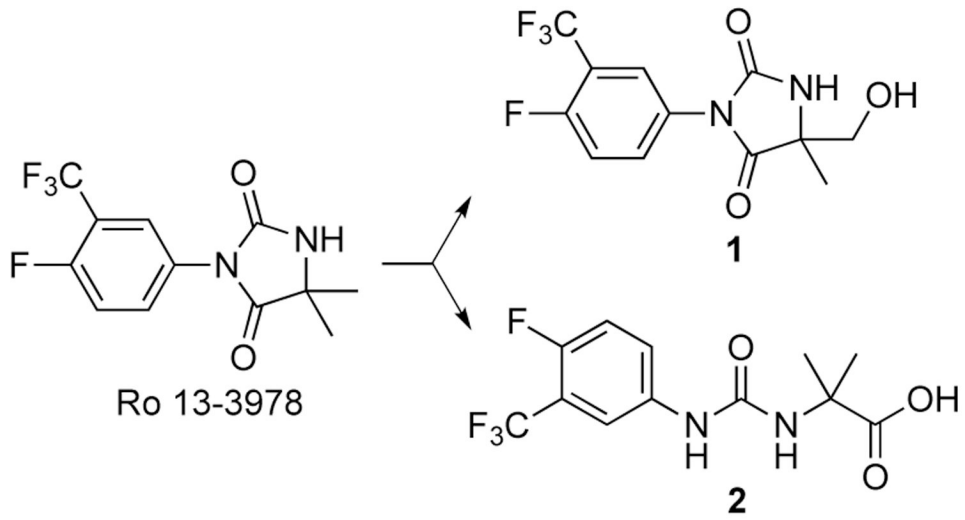


Figure 1.
Metabolism of Ro 13-3978

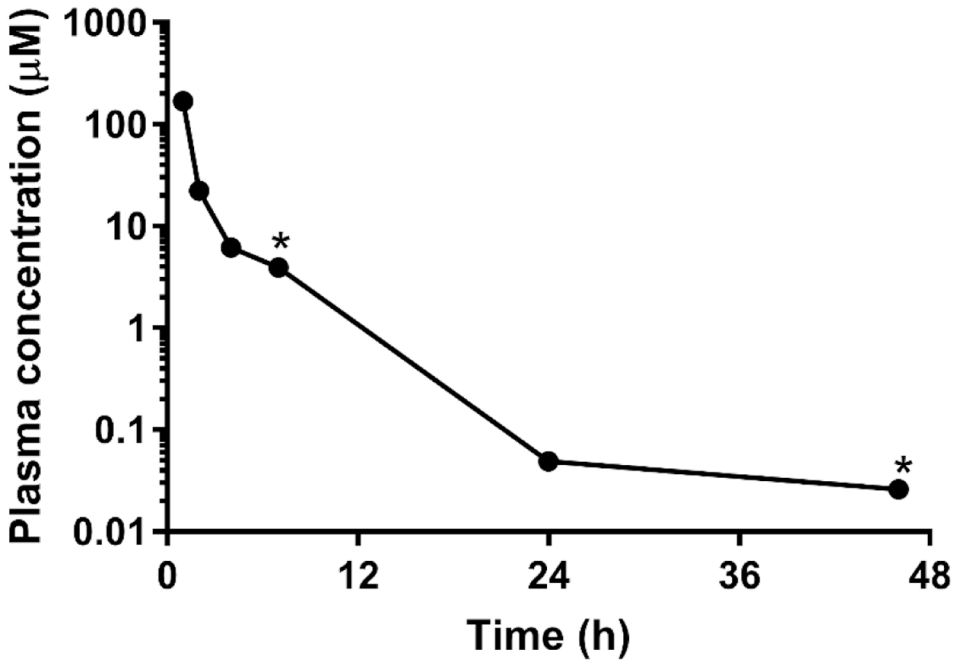
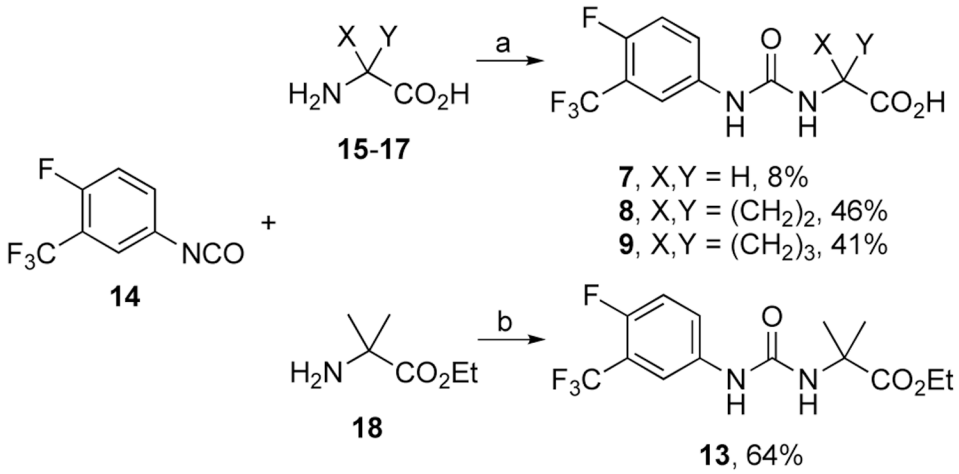


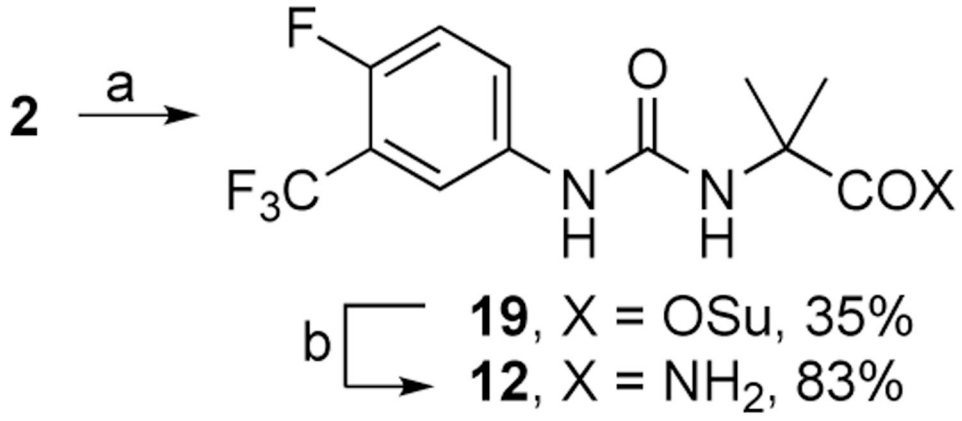
Figure 2.

Plasma concentration versus time profile for **2** following oral administration to mice at a dose of 100 mg/kg. Data points represent the mean of n=2 mice with the exception of the points marked with * (n=1).



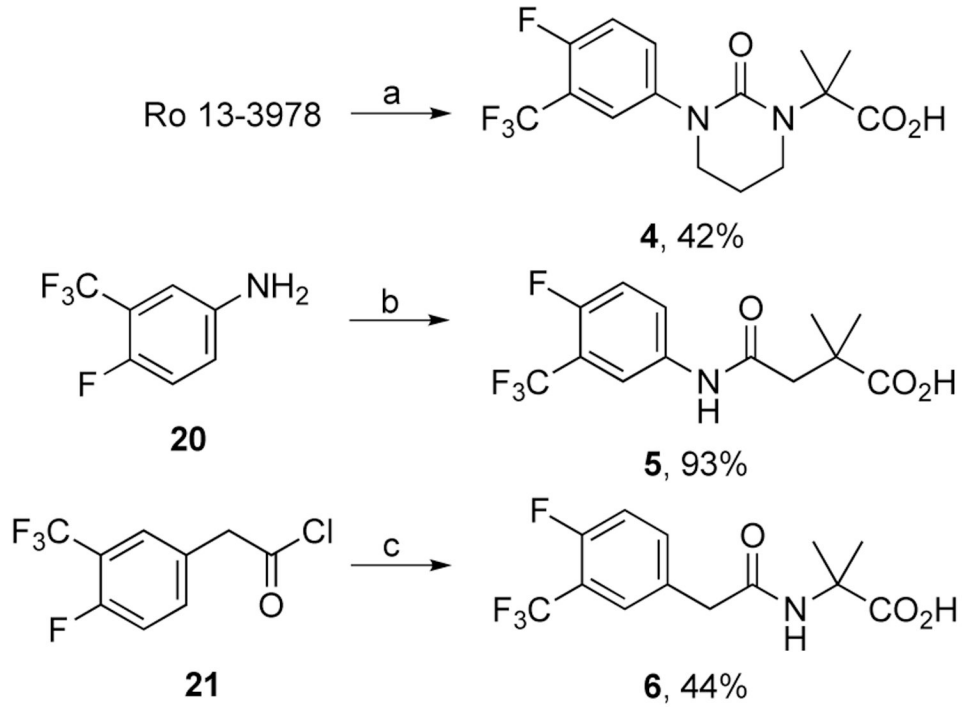
Scheme 1.

Reagents and conditions: (a) 1-2 M NaOH/CH_3CN, 0 °C to rt, 12 h, then pH to 3 with aq. HC1; (b) DIPEA, CH_2C1_2, 0 °C to rt, 12 h, then aq. HC1.



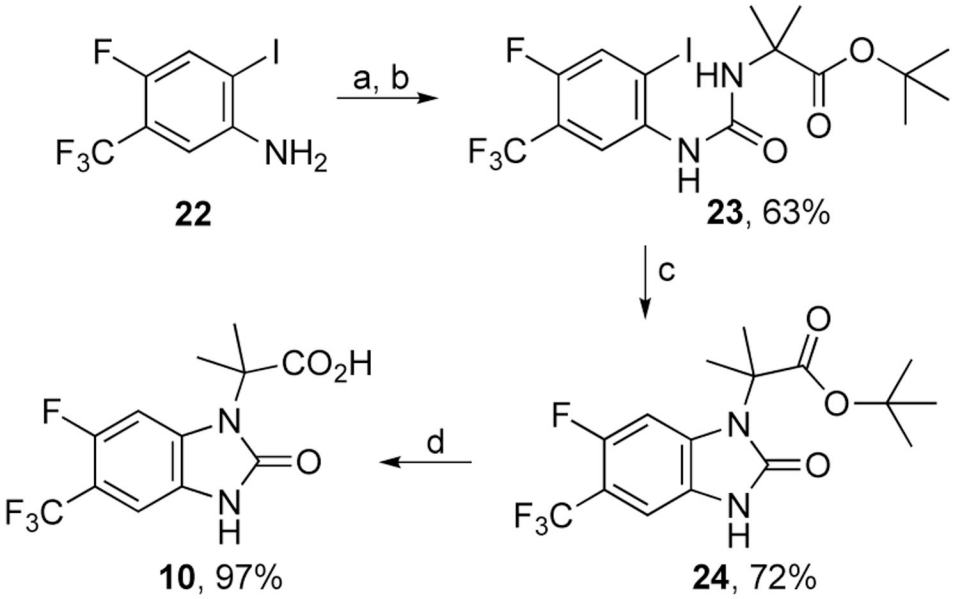
Scheme 2.

Reagents and conditions: (a) *N*-(3-dimethylaminopropyl)-*A*'-ethylcarbodiimide hydrochloride, *N*-hydroxysuccinimide, DMA, rt; 12 h; (b) 28% wt. NH_4OH, rt, 1.5 h.



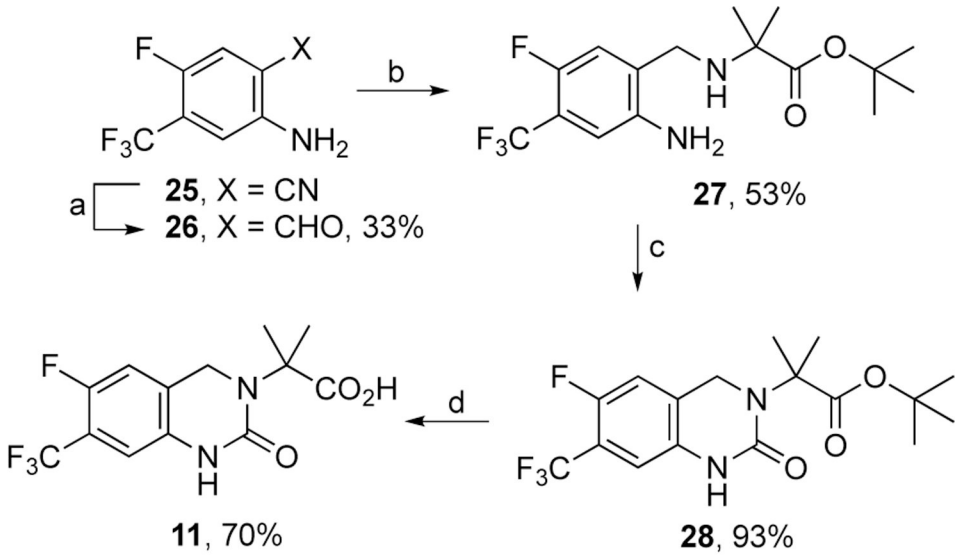
Scheme 3.

Reagents and conditions: (a) NaH, DMF, 0 °C to rt, 2 h then 1-bromo-3-chloropropane, rt, 48 h and pH to 3 with AcOH; (b) 3,3-dimethyldihydrofuran-2,5-dione, THF, rt, 12 h; (c) 2-amino-2-methylpropanoic acid, 2 M NaOH, 0 ° C, 2 h, then pH to 3 with aq. HCl.



Scheme 4.

Reagents and conditions: (a) triphosgene, toluene/EtOAc, 0-80 °C, 12 h; (b) *tert*-butyl 2-amino-2-methylpropanoate hydrochloride, DIPEA, CH_2Cl_2, 0 °C to rt, 12 h; (c) CuI, DBU, DMSO, 120 °C, 0.3 h; (d) TFA, CH_2Cl_2, rt, 24 h.

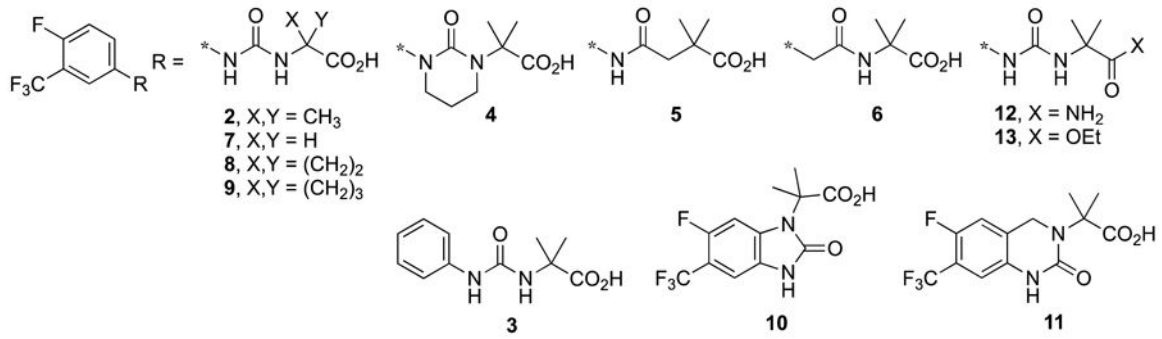


Scheme 5.

Reagents and conditions: (a) 1 M DIBAL in CH_2Cl_2, 0 °C to rt, 24 h; (b) *tert*-butyl 2-amino-2-methylpropanoate, AcOH, $NaCNBH_3$, rt, 7 days; (c) carbonyldiimidazole, dimethoxyethane, rt to reflux, 27 h; (d) TFA, CH_2Cl_2, rt, 24 h.

Table 1.

Physicochemical properties, in vitro metabolic stability and in vivo activity against *S. mansoni* for **2-13**.



Compd	$LogD_{7.4}$ [a]	PSA ($Å^2$) [a]	$Sol_{2.0}/Sol_{6.5}$ (μg/mL) [b]	h/m CL_{int} (μL/min/mg protein) [c]	*S. mansoni* WBR (%) 1 × 100 mg/kg po
2	−0.82	81.3	25-50/>100	<7/<7	67 [d]
3	−1.7	81.3	>100/50-100	<7/<7	20
4	−0.8	63.7	>100/>100	<7/<7	14
5	−0.12	69.2	25-50/25-50	<7/<7	42
6	−0.87	69.2	50-100/25-50	<7/<7	0
7	−1.9	81.3	>100/>100	<7/<7	49
8	−1.2	81.3	>100/>100	<7/<7	4
9	−0.7	81.3	50-100/>100	7/<7	21
10	−0.7	72.5	50-100/>100	<7/<7	0
11	−0.85	72.5	50-100/>100	<7/<7	0
12	1.8	84.2	50-100/50-100	19/18	38
13	3.1	67.4	3.1-6.3/3.1-6.3	ND [e]	70 [f]

[a] calculated using ChemAxon JChem for Excel

[b] Compounds in DMSO were spiked into either pH 6.5 phosphate buffer or 0.01 M HCl (approx. pH 2.0) and analyzed by nephelometry to determine a concentration range.

[c] in vitro intrinsic clearance measured in human and mouse liver microsomes

[d] data from Wang et al.[4]

[e] Metabolic stability parameters were not determined as rapid loss of compound was noted in control microsomal incubations in the absence of co-factor

[f] statistically different from control group (p=0.006)