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Bacterial translation inhibitors, 1-acylindazol-3-ols as anthranilic acid mimics

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Abstract—The discovery and initial optimization of a novel anthranilic acid derived class of antibacterial agents has been described in a recent series of papers. This paper describes the discovery of 1-acylindazol-3-ols as a novel bioisostere of an anthranilic acid. The synthesis and structure–activity relationships of the indazol bioisosteres are described herein.

We have previously reported the discovery of a novel series of antibacterial agents stemming from HTS hit compound **1**.[1] Such compounds were identified via a transcription/translation assay (T/T assay). In-depth structure–activity relationship studies were conducted using a combination of parallel and traditional synthesis leading to a number of highly active compounds, such as compound **2**. The acidic proton of the anthranilic acid portion of this class of compounds was shown to be critical for activity.[2] Analogs with the acid replaced with alternate groups such as sulfonamide, acetamide or hydrogen exhibited no activity versus *Staphylococcus aureus* (MICs >128 μg/mL).

Advanced compounds in this series failed to show activity in a standard mouse bacteremia model of infection, likely due to their high protein binding. Since human serum albumin is known to bind aromatic carboxylic acids such as salicylates and ibuprofen, we undertook a study to attempt to replace the carboxylic acid with bioisosteres to reduce the extent of protein binding. Traditional five-membered ring bioisosteres, such as tetrazole **3** (Fig. 1), were synthesized and were shown to retain activity similar to the corresponding parent carboxylic acid. However, protein binding of these bioisosteres was not significantly impacted.[2]

Since our interest in this series of novel translation inhibitors[3] was very high, we continued to try to develop replacements for the acid that improved potency and reduced the affinity for human serum albumin (HSA).[4,5]

Capillary electrophoresis measurement of the pK_a of this type of anthranilic acid indicates the presence of an intramolecular hydrogen bond, which pre-organizes the acid into a favorable bioactive conformation (Fig. 2, **I**).[6] It appeared to us that a 1-acylindazol-3-

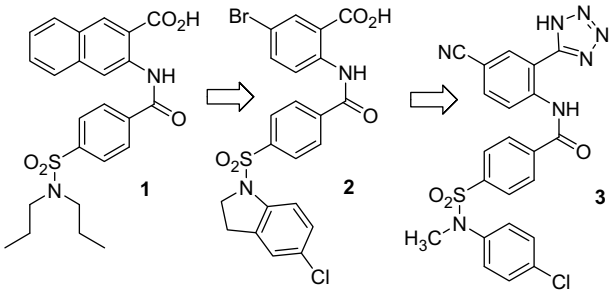

Figure 1. Initial SAR of HTS anthranilic acid screening lead **1**.

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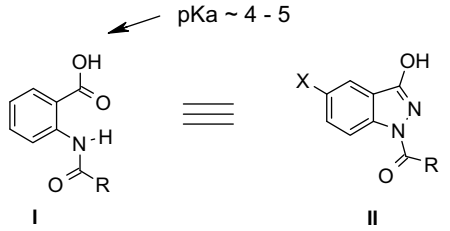


Figure 2. Comparison of indazole to anthranilic acid.

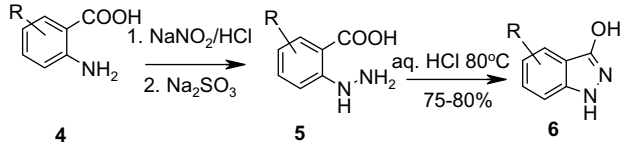
ols, **II** could closely approximate the desired conformation of the anthranilic acid, while still providing an acidic proton ($pK_a \approx 4$–5). Therefore, we hypothesized that conformational restraint in the form of an indazol would lead to a novel anthranilic acid bioisostere that may have improved properties. This report describes the synthesis and SAR of a series of novel indazol-3-ol translation inhibitor analogs.

1*H*-Indazol-3-ols (indazol, henceforth) are a well-known class of compounds and numerous syntheses have been reported.[7] The desired 5-substituted indazols are readily available via a single pot procedure starting from the corresponding anthranilic acid (Scheme 1). Diazotization of the aniline with sodium nitrite in hydrochloric acid is followed by reduction to the hydrazine. After heating the hydrazine with additional acid, the indazol is simply filtered away from the solution.

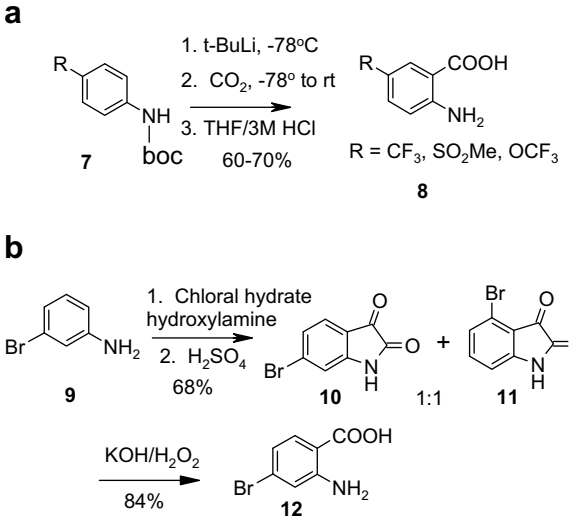
In a few instances the anthranilic acid was not commercially available and was synthesized from the aniline using one of two methods. The majority of the anthranilic acids were obtained through an *ortho*-directed lithiation and subsequent quenching with carbon dioxide (Scheme 2, route a). The 4-Br analog was synthesized via the isatin, given its incompatibility with the metallation conditions (route b).

The parent indazol compounds are potentially reactive at both of the ring nitrogens as well as the hydroxyl group, so regioselectivity was an issue when developing amide formation conditions. Conditions reported to limit reaction to the 1-position,[8] treatment of the cyano indazol **14** with an acid chloride in refluxing pyridine (Scheme 3), provided the desired analog in good yield. However, purification of the final product was troublesome, and when these conditions were applied to other analogs, multiple products formed (TLC).

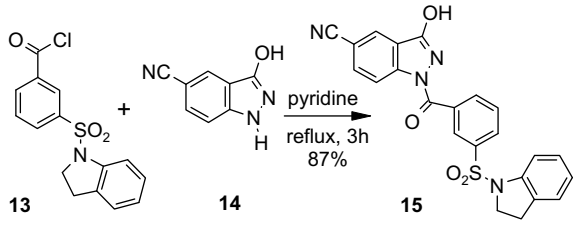
In order to resolve the problem of multiple products and to eliminate the need to form the acid chloride, a survey of conditions that would couple a carboxylic acid with



Scheme 1. Synthesis of indazol-3-ol fragment.



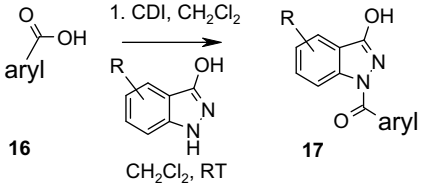
Scheme 2. Synthesis of anthranilic acids.



Scheme 3. First generation synthesis of 1-acylindazol-3-ols.

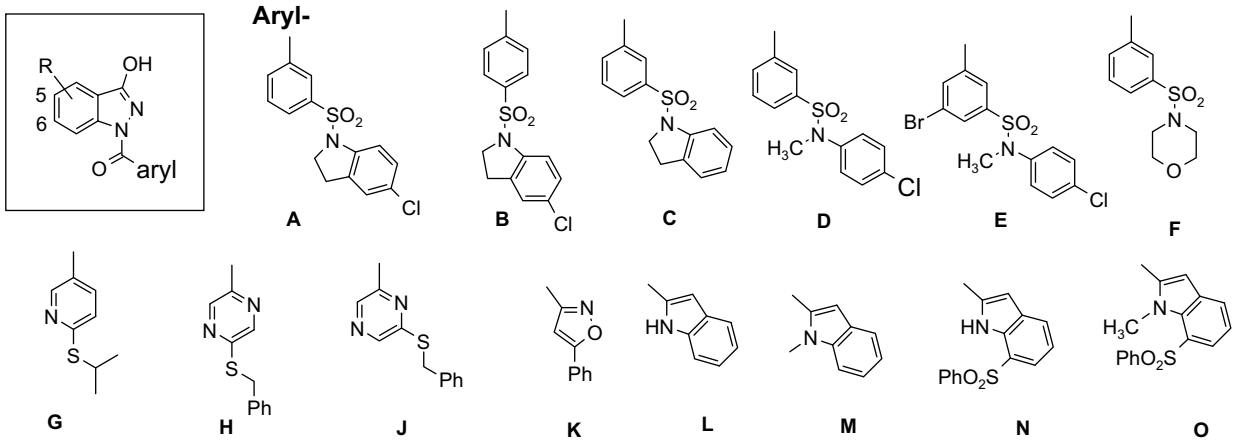
the indazol was performed. Fortunately, treatment of the acid (**16**) with 1,1′-carbonyldiimidazole (CDI), followed by the desired indazol, provided the coupled products **17** in good yields and typically as the sole products (Scheme 4).[9] Thirty analogs varying both the indazol ring substitution (9 indazols) and the aryl ring substitution (13 bottom pieces) were synthesized.[6] Selected compounds and their T/T inhibition and antibacterial activity are shown in Table 1.

Several of the analogs synthesized possessed good antibacterial potency such as compounds **19** and **47.** Compound **19**, in particular, exhibited potency that closely matched that of its parent anthranilic acid congener.[10] Many of the indazols synthesized exhibited modest antibacterial activity (e.g., **20**–**30**, **44**–**45**). Testing of antibacterial activity in the presence of serum indicated that these analogs were still highly protein bound. Even though the indazol core can successfully replace the anthranilic acid, it does not appear to offer any advantage in terms of reducing protein binding.



Scheme 4. Second-generation synthesis of 1-acylindazol-3-ols.

Table 1. Activity of compounds synthesized according to Scheme 4



Compound	R	Aryl	% T/T Inhibition at 100 μM[a]	SAUR MIC (μg/mL)[b]	SAUR MIC + 10% serum (μg/mL)[c]	Parent anthranilic acid MIC (μg/mL) (0% and 10% serum)
18	5-CN	B	5	64	>128	1, 64
19	5-CN	C	13	0.5	8	0.125, 8
20	5-Br	A	20	4	128	0.25, 64
21	5-Br	B	56	8	>128	1, 128
22	5-Br	C	52	8	128	1, 64
23	5-Cl	A	80	4	128	16, 128
24	5-Cl	B	61	16	>128	NT
25	5-Me	A	49	16	>128	16, 128
26	5-OCF_3	A	78	4	>128	NT
27	5-CF_3	A	62	8	>128	8, 128
28	6-Br	A	67	8	>128	32, 128
29	6-Cl	A	78	8	>128	NT
30	6-Cl	B	86	8	>128	NT
31	5-Br	D	55	>128	>128	1, 128
32	5-Br	E	29	>128	>128	0.25, 128
33	5-Br	F	3	>128	>128	4, 64
34	5-Cl	F	5	>128	>128	4, 64
35	5-CN	F	10	64	64	1, 8
36	5-Br	G	49	>128	>128	1, 32
37	5-CN	H	22	>128	>128	0.125, 4
38	5-Br	H	5	>128	>128	0.125, 16
39	5-Br	I	28	32	>128	16, 128
40	5-CN	I	7	>128	>128	64, 128
41	5-Br	J	42	16	>128	0.25, 16
42	5-CN	K	99	64	>128	2, 32
43	5-CN	L	49	64	>128	0.25, 4
44	5-CN	M	NT	16	>128	0.125, 1
45	5-Br	M	NT	16	128	0.125, 16
46	5-CN	N	NT	4	64	0.125, 1
47	5-Br	N	NT	2	128	0.125, 8

[a] *S. aureus* coupled transcription translation assay.[12]

[b] Minimum Inhibitory concentration versus *Staphylococcus aureus* UC9218.

[c] *Staphylococcus aureus* UC9218 + 10% Pooled Human Serum. Human serum (male, from Sigma) was thawed at room temperature, then placed in a 56 °C water bath for 30 min. The serum was filtered using a 0.2 micron filtration system.

In an attempt to better understand the SAR of the indazol series relative to the anthranilic acid series, small molecule X-ray crystal structures of an indazol analog **47** (Fig. 3) and a highly potent anthranilic acid analog (Fig. 4) were obtained.[11] It was hoped that the solid-state conformation would shed some light on the bound conformation. Interestingly, the structure of anthranilic acid **48** indicated that the northern two aromatic rings, the anthranilic acid and the indole ring, adopt a planar or nearly planar orientation (Fig. 4) because of the presence of an intramolecular hydrogen bond. In contrast, the indazol **47** (Fig. 3) is twisted significantly out of plane relative to the aromatic B-ring (Fig. 5). Compound **47** lacks the ability to form an intramolecular H-bond to the amide carbonyl because of the *N*-methyl indole substituent. Steric interactions between this

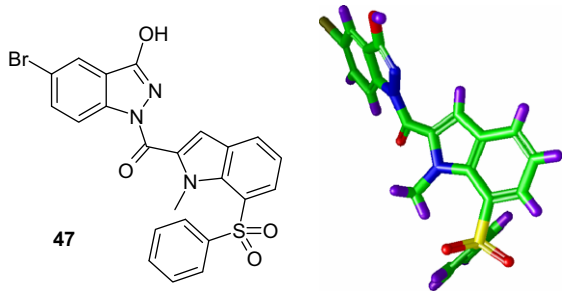


Figure 3. X-ray structure of indazol **47**.

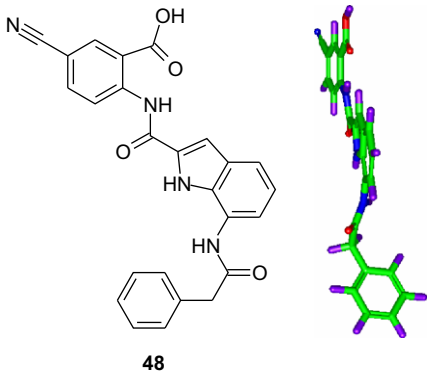


Figure 4. X-ray crystal structure of potent anthranilic acid **48**.

methyl substituent and the amide carbonyl may also cause the twisting. In contrast, anthranilic acids bearing indole N-1 methyl substitution (Table 1, analogs of compound **44** or **46**) are still highly potent. Modeling studies support the adoption of a planar conformation similar to compound **48** likely due to a steric interaction between the *N*-methyl indole substituent and the amide NH. The indazole ring may not provide enough bulk to bias the conformation. If the out of plane conformation of the indazols is the cause of their reduced activity relative to the anthranilic acid counterparts (Fig. 5), future indazol analogs should be designed with an aryl B-ring that can intramolcular H-bond to the indazol ring nitrogen or to the carbonyl of the amide. This would allow for the adoption of a planar conformation and subsequent proper positioning of the B-ring substituent.

The SAR of the indazol series of analogs deviates from that of the anthranilic series of analogs in one other way. Substitution at the 5-position of the anthranilic acid (corresponding to the 6-position on the indazol) is not well tolerated,[1] whereas in the indazol series the 6-substituted analogs are equipotent to the 5-substituted compounds (Table 1, **28** vs **20**, **29** vs **23**, **30** vs **24**). The indazol upper ring does not overlap exactly with the anthranilic acid when the southern half of the analog is held fixed (Fig. 5). This may cause the indazol to rotate or be positioned differently in the binding site allowing for this alternate substitution pattern.

In conclusion, we have discovered that an indazol ring can serve as a novel anthranilic acid bioisostere. Indazols are readily prepared via the synthetic routes outlined above. An X-ray structure of a potent anthranilic acid demonstrated that the upper two rings adopt a planar or nearly planar conformation (Fig. 2). In contrast, indazol analogs such as **47** (Fig. 1) are twisted out of plane, providing a hypothesis regarding their reduced activity relative to the anthranilic acids. Further investigation of the SAR around the acylindazol core and the B-ring structure with groups designed to favor a planar conformation may provide access to a more active series of compounds.

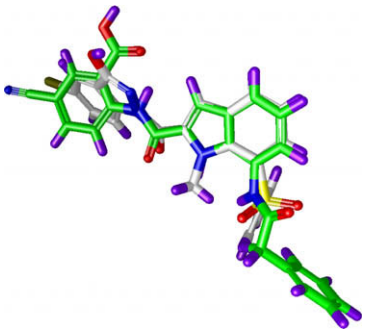


Figure 5. Structural overlay of 47 (white) and Anthranilic acid **48** (green).

References and notes

1. Unpublished results and Larsen, S. D.; Hester, M. R.; Ruble, J. C.; Kamilar, G. M.; Romero, D. L.; Wakefield, B.; Melchior, E. P.; Sweeney, M. T.; Marotti, K. R. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 6173.
2. Ruble, J. C.; Wakefield, B. D.; Kamilar, G. M.; Marotti, K. R.; Melchior, E. P.; Sweeney, M. T.; Zurenko, G. E.; Romero, D. L. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 4040.
3. Translation inhibition was demonstrated by using mRNA (no DNA) in the assay described in Ref. 12.
4. Li, J.; Wakefield, B. D.; Ruble, J. C.; Stiff, C. M.; Romero, D. L.; Marotti, K. R.; Sweeney, M. T.; Zurenko, G. E.; Rohrer, D. C.; Thorarensen, A. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 2347.
5. Thorarensen, A.; Wakefield, B. D. C. M.; Romero, D. L.; Marotti, K. R.; Sweeney, M. T.; Zurenko, G. E.; Rohrer, D. C.; Han, F.; Bryant, G. L. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 2823.
6. Stiff, C. M.; Zhong, M.; Sarver, R. W.; Gao, H.; Ho, A. M.; Sweeney, M. T.; Zurenko, G. E.; Romero*, D. L. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 2823.
7. Baiocchi, L.; Corsi, G.; Palazzo, G. *Synthesis* **1978**, 633.
8. Anderson, R. *J. Chem. Res., Synop.* **1985**, 376.
9. Compound **26**: 1-{3-[(5-chloro-2,3-dihydro-1H-indol-1-yl)sulfonyl]benzoyl}-5-(trifluoromethoxy)-1H-indazol-3-ol. Carboxylic acid (337 mg, 1.0 mmol) was placed in a scintillation vial and suspended in 10 mL of CH_2Cl_2. CDI was added (200 mg, 1.25 mmol) and the vial was capped and agitated for 4 h. Then the indazol (218 mg, 1.0 mmol) was added and the reaction mixture was allowed to agitate overnight (≈18 h). The reaction mixture was diluted with 50 mL of CH_2Cl_2. The organic layer was washed with 1 N HCl, water, and brine, and dried over Na_2SO_4. After concentration, the product was purified by flash chromatography (1% MeOH in CH_2Cl_2) affording 403 mg of an off-white solid (75% yield). Analytical sample obtained by recrystallization from MeOH. ^{1}H NMR

(400 MHz, DMSO) 2.96 (t, 2H), 4.02 (t, 2H), 7.24–7.26 (m, 2H), 7.47 (d, 1H), 7.73–7.77 (m, 2H), 7.86 (s, 1H), 8.01 (d, 1H), 8.22 (d, 1H), 8.37 (s, 1H), 8.47 (d, 1H), 12.45 (s, 1H). Anal. Calcd for $C_{23}H_{15}ClF_3N_3O_5S$: C, 51.36; H, 2.81; N, 7.81. Found: C, 51.41; H, 2.89; N, 7.79.

10. Antibacterial activity does not fully correlate with activity in the T/T assay, indicating that the compounds may operate by an additional mechanism. See: Mott, J. E.; Shaw, B. A.; Smith, J. F.; Bonin, P. A.; Romero, D. L.; Marotti, K. R.; Miller, A. A. *J. Antimicrob. Chemother.* **2008**, *62*, 720.
11. Crystallographic data (excluding structure factors) for the structures in the paper have been deposited at the Cambridge Crystallographic Data Centre as CCDC 646068 & 646069.
12. Murray, R. W.; Melchior, E. P.; Hagadorn, J. C.; Marotti, K. R. *Antimicrob. Agents Chemother.* **2001**, *45*, 1900.