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Tetrasubstituted pyrazinones derived from the reaction of praziquantel with *N*-bromosuccinimide

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Abstract

When praziquantel was exposed to *N*-bromosuccinimide in the presence of ethanol, a tricyclic 3-bromo-1-ethoxy pyrazinone was formed. From this and the analogous 1,3-dibromopyrazinone, a small library of 3-alkylamino-1-ethoxy, 1,3-dialkoxy, 3-alkoxy-1-bromo, and 3-alkylamino-1-bromo substituted pyrazinones were synthesized in high yields.

Keywords

praziquantel; *N*-bromosuccinimide; pyrazinone; piperazinone

Praziquantel (**1**) is the drug of choice for schistosomiasis.^{1,2} One of the few liabilities of **1** is its rapid first-pass drug metabolism^{2–4} to form a number of metabolites, including the major *trans*-cyclohexanol metabolite **2**^{5,6} that is reported to be equal to or considerably less effective than the parent drug (Figure 1).^{7–9} As **1** is so rapidly metabolized, it is reasonable to assume that other yet to be identified metabolites might also contribute to its antischistosomal activity. In this respect, structures for these putative metabolites have not been confirmed, but biomimetic oxidation¹⁰ and human pharmacokinetic and CYP450 studies^{3,11,12} have identified two to seven monohydroxy, four dihydroxy, one trihydroxy, and one to two dehydrogenated metabolites; the latter may have arisen by dehydration reactions of monohydroxy metabolites. Interestingly, **3**, the *gem*-difluoro analog of **1**, is only marginally more metabolically stable than the latter, but it has lower antischistosomal activity; however, unlike **1**, **3** has activity against the juvenile stage of this parasite.¹³ In

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Supplementary data

Supplementary data (compound preparation and characterization) data associated with this article can be found in the online version, at

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sum, these data suggest that further studies to sort out the metabolic ambiguities of **1** might lead to new insights into its mechanism of action and provide new directions for drug discovery. We now report the outcome of our initial experiments to access the yet to be identified metabolites of **1** via bromination reactions using *N*-bromosuccinimide (NBS).

When we exposed **1** to NBS in chloroform solvent, a proton NMR of the crude reaction mixture indicated formation of a product with an ethoxy substructure. We surmised that the 0.75% EtOH in the chloroform had participated at some point in the reaction sequence. We then ran a reaction with a 1:3:2 molar ratio of **1**:NBS:EtOH in dichloromethane and isolated pyrazinone **6** (3-bromo-1-ethoxy-6,7-dihydro-4*H*-pyrazino[2,1-*a*]isoquinolin-4-one) in 38% yield (Scheme 1). We also observed concomitant formation of **7**, the ethyl ester of cyclohexane carboxylic acid, with GC-MS and in NMR spectra. Attempts to improve the yield of **6** by running the reaction in pure EtOH or by replacing the dichloromethane cosolvent with acetonitrile were unsuccessful.

We propose that **6** arose from an initial α -bromination of **1** to form intermediate **4** (Scheme 1), a reaction that has precedent to α -brominations of structurally related 2,5-diketopiperazines with NBS.¹⁴ This was followed by attack of EtOH on the exocyclic amide of **4** or its corresponding acyliminium ion intermediate¹⁵ to form conjugated imine **5**. We surmised that the conversion of **1** to **4** was reversible since **1** was inert to NBS in dichloromethane solvent (without EtOH). Evidently, further bromination-dehydrobromination reactions must have ensued to account for the formation of **6**. However, followup experiments showed that neither pyrazinones **8** nor **10** were reaction intermediates in this sequence, as no **6** was formed from treatment of either compound with a mixture of NBS and EtOH in dichloromethane. Pyrazinone **8** was synthesized independently by treatment of **1** with 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ),¹⁶ and **10** was synthesized in 32% yield by reaction of piperazinone **9**¹³ with NBS.

1D and 2D NMR data for **6** identified this reaction product as a tricyclic pyrazinone, but the weak NOE correlations for the ethoxy substructure did not distinguish between the assigned 3-bromo-1-ethoxy (**6**) and alternate 1-bromo-3-ethoxy (**14**) regioisomers (Figure 2). As described below in Scheme 2, pyrazinone **14** was synthesized independently from **10**, and its melting point and NMR spectra differed from that of **6**. However, definitive assignment of the structure of pyrazinone **6** was possible only by key HMBC correlations and ROESY NOE's in the 2D NMR data of its derivative **17** (Figure 2), obtained as described below in Scheme 2. These included HMBC correlations from the CH₂ hydrogen atoms α to the NH to C-3, from the NH and 6-CH₂ hydrogen atoms to C-4, and from the OCH₂ hydrogens on the C-1 ethoxy substructure to C-1. ROESY NOE's from H-11 to H-10 and to both sets of hydrogens of the C-1 ethoxy substituent clinched the regioisomer assignment.

Clearly, bromination of **1** with NBS was not going to provide a path forward to access metabolites of **1**, all of which contain its cyclohexanecarboxamide substructure. Nonetheless, we decided to investigate the chemistry of **6** and **10** with the aim of accessing novel fully substituted pyrazinones (Scheme 2). First, we found that **6** could be easily converted to 1,3-dialkoxy pyrazinones **11** and **12** by treatment with the corresponding alkoxide/alcohol combinations. However, **6** did not react with the *t*-butyl alkoxide/*t*-butyl

alcohol combination. Similarly, reaction of **6** with a variety of primary and secondary amines afforded the 3-alkylamino-1-ethoxy pyrazinones **15–20** in yields > 80%. These reactions are similar to those described for other 3-bromo- and 3-chloropyrazinones.^{17,18} A parallel set of reactions with 1,3-dibromopyrazinone **10** (Scheme 2) led to reaction products with substitution occurring exclusively at the imidoyl bromide (position 3). These are exemplified by 3-alkoxy-1-bromo pyrazinones **13** and **14** and 3-alkylamino-1-bromo pyrazinones **21–26** which were all produced in high yields. Interestingly, other dibromopyrazinones react with anilines under acidic (camphorsulfonic acid) conditions with the same regioselectivity.¹⁹ Finally, pyrazinone **27** was obtained in 80% yield by reductive debromination of **24** with hydrogen and a nickel catalyst. For all of these pyrazinone syntheses, the reaction products were isolated by simple precipitation.

In summary, we synthesized a small library of fully substituted pyrazinones²⁰ that may have some application in drug discovery. For **15–27**, predicted²¹ Log P values ranging from 1.2 to 3.2 and calculated²² polar surface area (PSA) values ranging from 36 to 74 Å indicate that these pyrazinones have physicochemical profiles favorable for compound optimization. We note that the pyrazinone heterocycle appears to be quite chemically and metabolically stable, and that structurally diverse 3-aminopyrazinones possess a variety of biological activities.^{17–19} In this respect, we tested **15–27** for cytotoxicity and for activity against a panel of protozoan parasites including *Trypanosoma cruzi*, *Leishmania donovani*, *Plasmodium falciparum*, and *Trypanosoma brucei rhodesiense*. None of the pyrazinones had notable activity against *T. b. rhodesiense* (IC_{50s} >70 µM) or *T. cruzi* (IC_{50s} >23 µM). However, representative data (Table) showed that **19**, with an IC₅₀ value of 1.7 µM against *L. donovani*, was only 5-fold less potent than the control drug miltefosine against this protozoan parasite. Pyrazinone **19** had minimal activity against the other protozoan parasites, and it had low cytotoxicity (IC₅₀ 160 µM). Similarly, **23** had single digit micromolar activity against *P. falciparum*, and it also had low cytotoxicity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Figure 2.

Scheme 1.

Reagents and conditions: (a) NBS, EtOH, CH₂Cl₂, rt, 24 h; (b) NBS, CH₂Cl₂, rt, 24 h.

Scheme 2.

Reagents and conditions: (a) R₁Na, ROH, rt, 12 h; (b) R₁R₂NH, THF, rt, 12 h; (c) H₂, Ni, aq. NaOH, MeOH/THF, rt, 24 h.

Table

Antiprotozoal activity and selectivity of selected pyrazinones.

Compound	IC ₅₀ (μM)		
	<i>L. donovani</i> ^a	<i>P. falciparum</i> ^b	cytotoxicity ^c
12	130	55	68
18	150	78	250
19	1.7	74	160
23	18	6.6	140
Miltefosine	0.34	ND	ND
Chloroquine	ND	0.15	ND
Podophyllotoxin	ND	ND	0.0072

^a MHOM-ET-67/L82 strain^b K1 strain^c rat skeletal myoblast L6 cell line