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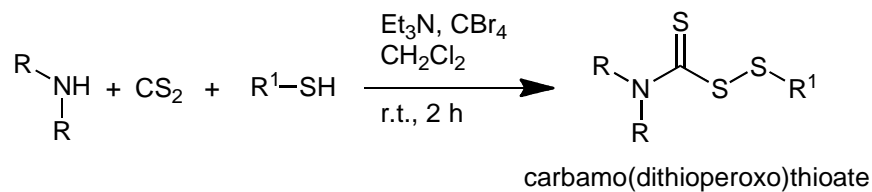To create your abstract, type over the instructions in the template box below.
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Tetrahedron Letters
journal homepage: www.elsevier.com

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ARTICLE INFO

ABSTRACT

A new, simple, one-step synthetic route to carbamo(dithioperoxo)thioates from commercially available starting materials is described. The key step of this new synthetic approach involves the tetrabromomethane-promoted reaction between secondary amines, carbon disulfide and alkyl thiols under basic conditions at room temperature. New compounds from this series selected for anticancer screening showed selective sub-micromolar activity within BCA2-expressing human breast cancer cell lines.

The thiuram disulfide, disulfiram (Antabuse®, Figure 1) is a well-established drug used in the treatment of alcoholism (aversion therapy) by virtue of its aldehyde dehydrogenase (ALDH) inhibitory activity.[1] Previous studies within our group have identified disulfiram as a zinc-binding inhibitor of the E3 ubiquitin ligase enzyme, BCA2 (Breast Cancer Associated protein 2),[2] a clinically over-expressed protein in breast cancer patients and a target for cancer drug discovery.[3] The interesting and multi-faceted anticancer activity of disulfiram, coupled with its favourable clinical toxicological and safety profile, has led to the initiation of several cancer clinical trials using this agent.[4]

Figure 1. Chemical structures of disulfiram and carbamo(dithioperoxo)thioates as potential metabolites.

The *in vivo* pharmacokinetic profile of disulfiram in patients is complex and is incompletely understood. The initial cleavage of the weak disulfide bond to form the corresponding diethyldithiocarbamate anion (known as ditiocarb) is well established. It has been postulated that the complex of ditiocarb with copper(II) ions (CuEt) formed in the blood is likely to be responsible for the anticancer activity of disulfiram.[5] An alternative reported pharmacokinetic pathway involves the formation of carbamo(dithioperoxo)thioates (mixed disulfides derived from disulfiram, Figure 1) following initial formation of ditiocarb. Notably carbamo(dithioperoxo)thioates have been reported as inhibitors of human mitochondrial[6] and sheep liver[7]

ALDH, the therapeutic target on which clinical approval of disulfiram is based.

During previous work investigating the BCA2-inhibitory activity of disulfiram analogues, we synthesized a small series of carbamo(dithioperoxo)thioates by heating a mixture of disulfiram (or related piperidinyl or pyrrolidinyl analogues) with alkyl thiols in ethanol under reflux (Scheme 1).[2] This procedure led to the formation of the required carbamo(dithioperoxo)thioate products in low yields (9-25%) following extensive column chromatography, alongside a number of mixed disulfide-based by-products.

Scheme 1. Previously reported synthesis of carbamo(dithioperoxo)thioates by reaction of disulfiram analogues with alkyl thiols.

Testing of new disulfiram analogues and the structurally related carbamo(dithioperoxo)thioates in human breast cancer cell lines with differing levels of expression of BCA2, including isogenic cell lines MDA-MB-231 (BCA^{low/-}) and MDA-MB-231/ER (BCA2^{+}), revealed interesting results.[2] Of particular note were the carbamo(dithioperoxo)thioates derived from reaction of disulfiram with hexanethiol or 2-mercaptoethanol (Figure 1; R = *n*-hexyl, $HOCH_2CH_2$). Both of these analogues exhibited sub-micromolar IC_{50} activity selectively in BCA2-expressing breast cancer cell lines, whilst lacking the ALDH-inhibitory activity characteristic of the parent compound disulfiram.

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Given the biological relevance of the carbamo(dithioperoxo)thioates in the context of anticancer drug discovery, and the difficulties in obtaining pure products through the previously described mixed disulfide method,[2] we required a new synthetic protocol for reliable and high yielding access to this class of compound. In this paper, we describe a simple, one-step protocol for the synthesis of a range of substituted carbamo(dithioperoxo)thioates under mild conditions. Our new method is based on the procedure developed by Liang and co-workers, using tetrabromomethane (CBr$_4$) to promote the synthesis of thioureas and thiuram disulfides via reaction of amines with carbon disulfide.[8] The use of carbon tetrabromide to promote the reaction of dithioic acids or thiols with active methylene compounds to produce dithiocarbamates, xanthates, dithioesters and thioethers has also been previously reported.[9] Critical to these studies was the proposed generation of a sulfenyl bromide intermediate.

We now report the extension of the above studies to the synthesis of carbamo(dithioperoxo)thioates. Initially, we optimized the reaction conditions based on the reaction between diethylamine, carbon disulfide, 2-mercaptoethanol or hexanethiol, and triethylamine as the base, to investigate the formation of the previously described lead compounds shown in Figure 1 (R = n-hexyl, HOCH$_2$CH$_2$). As a first adaptation of the previously reported method,[8] the use of equimolar quantities of reagents without base in DMF as the solvent at room temperature gave very low yields of product. Reactions of equimolar quantities of these reagents promoted by triethylamine in dichloromethane in the absence of CBr$_4$ did not lead to the desired carbamo(dithioperoxo)thioate products. Adding one equivalent of CBr$_4$ in dichloromethane produced the products in 48% and 38% yields, respectively. Surprisingly, the use of two equivalents of alkyl thiol did not lead to the desired products, instead yielding the corresponding dithiocarbamates (confirmed by mass spectrometry and NMR) as by-products. Finally, it was found that the use of two equivalents of CBr$_4$ gave the products in good yields (85% and 58%, respectively) under mild conditions (room temperature, 2 h reaction time). This optimized procedure was subsequently adopted for the synthesis of new carbamo(dithioperoxo)thioate analogues.

Following mixing of equimolar quantities of secondary amine (**1a-f**) and alkyl thiol (**2a-d**) in anhydrous dichloromethane, the mixture was cooled in an ice bath. Carbon disulfide (1 equiv) was then added dropwise, followed by slow addition of triethylamine (1.1 equiv). After a further five minutes, a solution of CBr$_4$ (2 equiv) in dichloromethane was added, followed by stirring at room temperature for two hours. Product work-up and purification by column chromatography gave carbamo(dithioperoxo)thioates **3a-x** (>60% yield in most cases) (Scheme 2).

Scheme 2. Optimized synthesis of carbamo(dithioperoxo)thioate analogues **3a-x**.

The identity of the carbamo(dithioperoxo)thioate products was confirmed by NMR spectroscopy and mass spectrometry. Compound purity following column chromatography was further verified by either microanalysis (duplicate % CHN) or accurate mass determination (see Supplementary data). Good yields were obtained for the majority of products, however, in the case of compounds **3q** and **3u** (derived from 2-mercaptoethanol and

morpholine or N-methylpiperazine), lower yields (25% and 30% respectively) were obtained. Overall, our results are consistent with the formation of an intermediate sulfenyl bromide, as was postulated in the previous related studies.[8,9]

As a continuation of our previously published studies on the anticancer properties of these new disulfiram-derived molecules,[1,2] we carried out a preliminary in vitro evaluation across human breast cancer cell lines where BCA2 expression status was known (72 h compound treatment time). The mean IC$_{50}$ values (triplicate analysis) for compounds **3i**, **3k**, **3o**, **3s** and **3w** (derived from a variety of secondary amines) in human breast cancer cell lines MCF-7 (BCA2-positive), MDA-MB-231 (BCA2-negative) and MCF-10A (non-cancerous control) were determined. The results presented in Table 1 indicate selective sub-micromolar IC$_{50}$ activity selectively within MCF-7 cells, consistent with previously synthesized analogues of this broad class.[2] One analogue (**3o**) possessed a biphasic dose-response profile, a phenomenon previously observed within our group for disulfiram.[1]

In conclusion, we have developed and optimized a new one-pot synthetic route to a range of potential anticancer carbamo(dithioperoxo)thioates in high yields, including the previous BCA2-inhibitory compounds from our previous study (compounds **3a** and **3c** in 58% and 85% yields, respectively). Further studies of the anticancer properties of this new series are ongoing.

Acknowledgments

Supplementary Data

Supplementary data (experimental procedures and characterization data, including NMR and mass spectrometry data for all isolated compounds, and the in vitro testing protocol) associated with this article can be found in the online version.

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Compound	MCF-7	MDA-MB-231	MCF-10A
3i	0.5	>10	>10
3k	0.9	>10	>10
3o	0.6[a]	>10	>10
3s	1	10	>10
3w	0.9	>10	>10

The results are the mean values from three independent experiments; [a] indicates the first IC_{50} value of a biphasic dose-response profile.

Table 1. IC_{50} values (μM) in human breast cancer cell lines MCF-7 (BCA2 +ve), MDA-MB-231 (BCA2 –ve) and MCF-10A (control proliferative cell line).
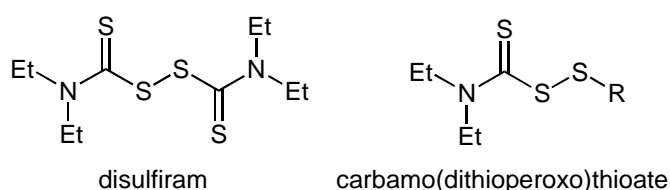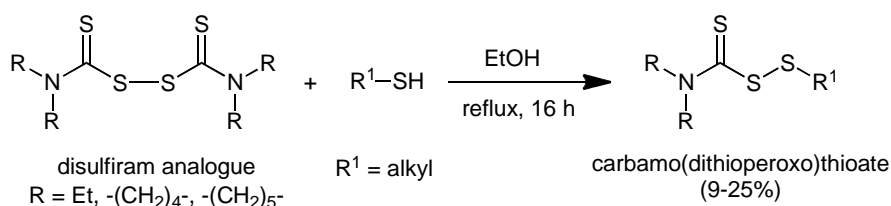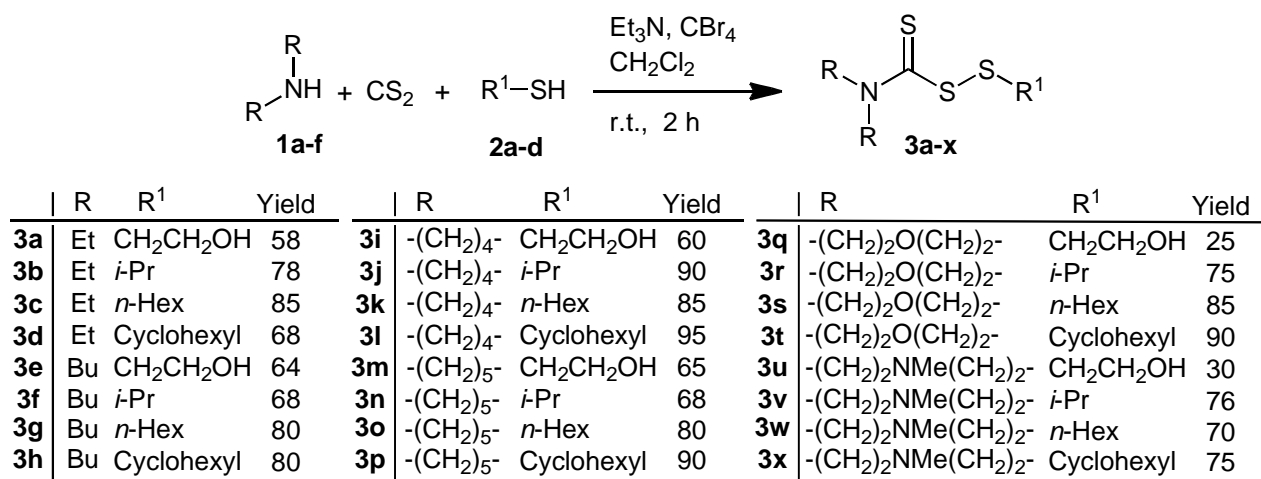

Figure 1. Chemical structures of disulfiram and carbamo(dithioperoxo)thioates as potential metabolites.

Scheme 1. Previously reported synthesis of carbamo(dithioperoxo)thioates by reaction of disulfiram analogues with alkyl thiols.

	R	R^1	Yield		R	R^1	Yield		R	R^1	Yield
3a	Et	CH$_2$CH$_2$OH	58	**3i**	-(CH$_2$)$_4$-	CH$_2$CH$_2$OH	60	**3q**	-(CH$_2$)$_2$O(CH$_2$)$_2$-	CH$_2$CH$_2$OH	25
3b	Et	*i*-Pr	78	**3j**	-(CH$_2$)$_4$-	*i*-Pr	90	**3r**	-(CH$_2$)$_2$O(CH$_2$)$_2$-	*i*-Pr	75
3c	Et	*n*-Hex	85	**3k**	-(CH$_2$)$_4$-	*n*-Hex	85	**3s**	-(CH$_2$)$_2$O(CH$_2$)$_2$-	*n*-Hex	85
3d	Et	Cyclohexyl	68	**3l**	-(CH$_2$)$_4$-	Cyclohexyl	95	**3t**	-(CH$_2$)$_2$O(CH$_2$)$_2$-	Cyclohexyl	90
3e	Bu	CH$_2$CH$_2$OH	64	**3m**	-(CH$_2$)$_5$-	CH$_2$CH$_2$OH	65	**3u**	-(CH$_2$)$_2$NMe(CH$_2$)$_2$-	CH$_2$CH$_2$OH	30
3f	Bu	*i*-Pr	68	**3n**	-(CH$_2$)$_5$-	*i*-Pr	68	**3v**	-(CH$_2$)$_2$NMe(CH$_2$)$_2$-	*i*-Pr	76
3g	Bu	*n*-Hex	80	**3o**	-(CH$_2$)$_5$-	*n*-Hex	80	**3w**	-(CH$_2$)$_2$NMe(CH$_2$)$_2$-	*n*-Hex	70
3h	Bu	Cyclohexyl	80	**3p**	-(CH$_2$)$_5$-	Cyclohexyl	90	**3x**	-(CH$_2$)$_2$NMe(CH$_2$)$_2$-	Cyclohexyl	75

Scheme 2. Optimized synthesis of carbamo(dithioperoxo)thioate analogues **3a-x**.