



Introduction:

Mebendazole, a member of the benzimidazole family (Fig. 1), is a microtubule-disrupting anthelmintic drug that is used extensively for gastrointestinal parasitic infections in humans. Marketed status, oral availability and a favorable safety profile in long-term treatment regimens¹ made mebendazole a good target for drug repositioning when the antineoplastic properties of the compound were discovered.

Mebendazole inhibited melanoma growth at pharmacological concentrations². It was hypothesized that this effect occurred by inducing apoptosis in melanoma cells through phosphorylation of Bcl-2. Phosphorylation of Bcl-2 can prevent its interaction with the proapoptotic factor Bax, thereby promoting apoptosis.

This proposed mechanism of action is controversial however. It has also been suggested that mebendazole, as a microtubule-damaging agent, inhibits normal spindle formation and causes mitotic arrest, followed by apoptotic cell death, caspase activation, and cytochrome c release³.

More than a dozen articles have been published showing the antitumor properties of mebendazole against a variety of cancers^{4,5}. Most researchers agree, however, that the exact mechanism of action of mebendazole has yet to be elucidated, because the drug's action differs from "traditional" microtubule-targeted drugs and the exact cellular events leading to phosphorylation of Bcl-2 are still unclear.

Objective:

To investigate a possible alternative use for mebendazole in cancer, and to evaluate possible mechanisms of antineoplastic action using the *in silico* structure evaluation tools in MetaDrug™ (GeneGo, Inc., Encinitas, CA).

Methods:

Mebendazole was uploaded into MetaDrug™ as a .mol structure file, and all default settings were left unchanged, allowing for phase I metabolite prediction, calculation of physical properties (e.g. Rule of 5⁶) for the uploaded compound, and target identification through similarity search (Tanimoto coefficient of 0.7⁷).

Enrichment analysis⁸ in MetaDrug was used to study predicted targets by GeneGo Pathway Maps and GeneGo Disease Biomarker Networks.

Results and Discussion:

At a Tanimoto similarity level of 0.7, MetaDrug identified eight potential targets for mebendazole. Tubulin was predicted as a primary target based on mebendazole's similarity to the close analog nocodazole and to compound B (Fig. 1). A possible influence of mebendazole on transcription factors participating in myogenesis, myogenin, MEF2D, and AhR, was also predicted through nocodazole.





Interestingly, MetaDrug predicted inhibition of baculoviral IAP repeat-containing protein 4 (XIAP) and baculoviral IAP repeat-containing protein 3 (c-IAP2) belonging to the Inhibitor of Apoptosis Protein family of anti-apoptotic proteins. These targets were predicted through similarity to compound C (Fig 1).

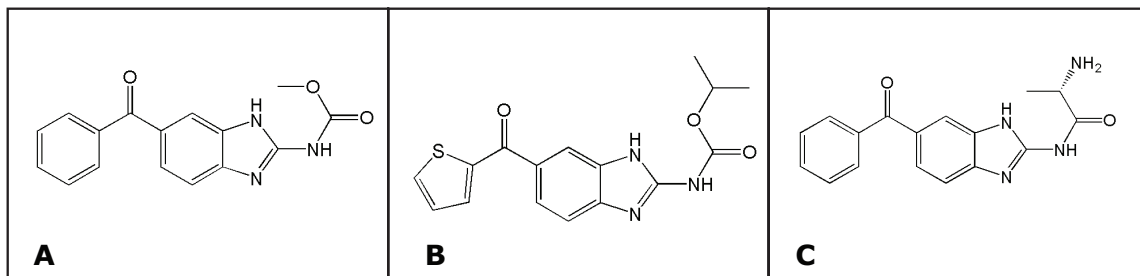


Figure 1. Mebendazole (A) and similar compounds in MetaDrug. Tubulin was identified as a target of both nocodazole (similarity 0.75) and compound B (similarity 0.71). XIAP and c-IAP2 were identified as targets of compound C (similarity 0.82).

Next, enrichment analysis on the list of predicted targets was performed. GeneGo Pathway Maps enrichment histogram (Fig. 2) shows that apoptosis and Cell survival-related pathways were the most impacted pathways affected by the predicted targets of mebendazole.

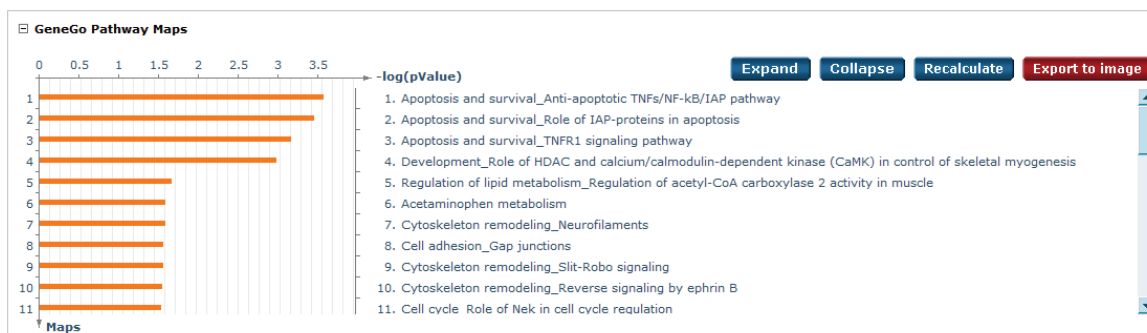


Figure 2. GeneGo Pathway Maps enrichment histogram.

Enrichment analysis of predicted targets using the GeneGo Disease Biomarker Networks ontology (Fig. 3) suggests that mebendazole may have antineoplastic activity. This activity has been shown in mouse model of lung cancer. Oral administration of mebendazole to mice elicited a strong antitumor effect in a model of lung cancer and reduced lung colonies in experimentally induced lung metastasis³.

In order to investigate the mechanism of action of mebendazole, the role of its predicted targets in cellular pathways was studied using GeneGo Pathway Maps. Descriptions of the maps Apoptosis and survival_Role of IAP-proteins in apoptosis (not shown) and Apoptosis and survival_FAS signaling cascades (Fig. 4) indicate that XIAP is a potent inhibitor of active caspases 9 and 3. By blocking active caspase 3, XIAP inhibits the downstream portion of the caspase cascade and thus blocks apoptosis triggered by multiple caspase activation pathways⁹.

Over-expression of XIAP in malignant cell lines induces chemoresistance¹⁰, and in some studies of patients with malignancies, increased XIAP expression is associated with poor outcome¹¹. Given these observations and the biological role of XIAP in apoptosis, there is an interest in developing XIAP inhibitors as therapeutics. Several inhibitors of XIAP have been described in the literature^{4,5}. Constitutive caspase-9 and caspase-3 activation^{12,13}, and activation of JNK and ERK¹³ were shown in cells treated with XIAP-inhibitors. Phosphorylation of Bcl-2 was also described in mebendazole-treated cells (see above).

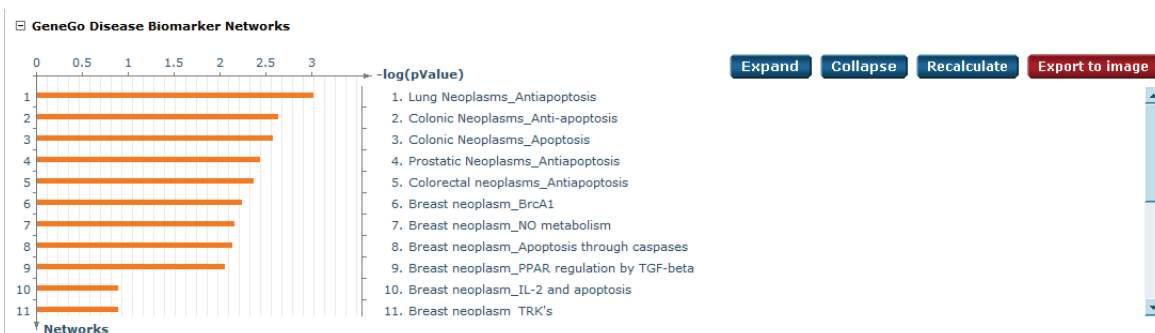


Figure 3. GeneGo Disease Biomarker Networks enrichment histogram.

Taking into account these observations, the GeneGo map Apoptosis and survival_FAS signaling cascades, which illustrates the roles of XIAP, caspase-3, caspase-9 and Bax in apoptosis, suggests the following mechanism of action for mebendazole. XIAP inhibition leads to caspase-9 and caspase-3 activation that blocks antiapoptosis and induces apoptosis development (Fig. 4). Caspase-3 activates PAK2 kinase which leads to subsequent activation of MEK4, MEK1 and JNK kinases. The latter phosphorylates Bcl-2, preventing its inhibitory interaction with the proapoptotic Bax, thereby promoting apoptosis.

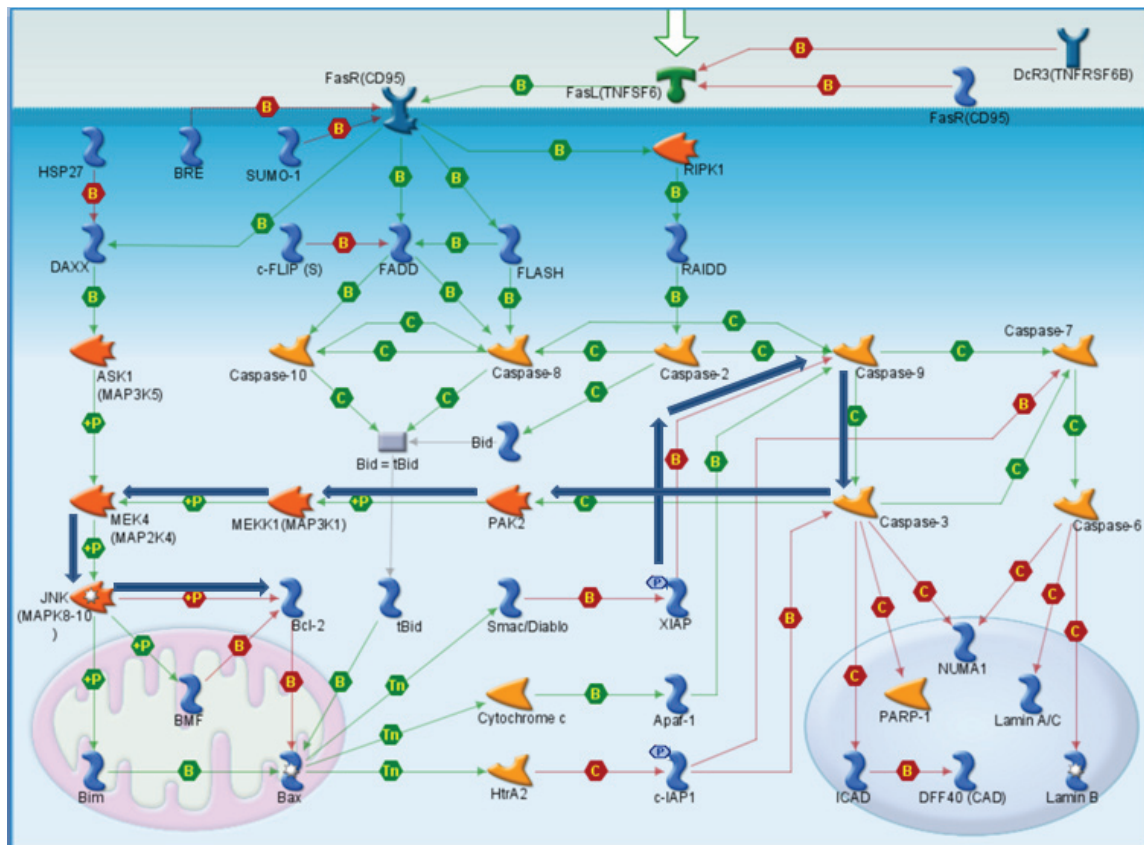


Figure 4. Apoptosis and survival_FAS signaling cascades canonical pathway map. Blue arrows indicate the suggested mechanism of action for mebendazole.



Conclusions:

A new potential therapeutic use as an antineoplastic agent was identified for the anthelmintic drug mebendazole by comparison of the compound to structurally-related molecules in MetaDrug. MetaDrug also predicted a mechanism for this activity via inhibition of XIAP. This suggested mechanism of action is supported by observations in XIAP-inhibited cells. The proposed inhibition of XIAP by mebendazole should be further investigated to confirm its involvement in the compound's antineoplastic effects.

References:

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