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Meclonazepam analogues as potential new antihelmintic agents

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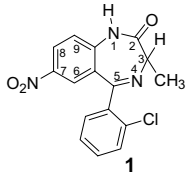
Abstract—New analogues of the potent antihelmintic meclonazepam were prepared and evaluated against *Schistosoma mansoni*. The biological data suggests substitution at positions 2 and 4 of meclonazepam could provide promising analogues for prophylactic and therapeutic activity against *S. mansoni*.

Infectious diseases such as Schistosomiasis spread by parasites are the causative agents of several highly disabling and often fatal human diseases especially in the tropical and subtropical areas.[1] These parasitic infections require a mammalian host which is indispensable for maintaining the parasite. There are limitations associated with currently available drugs available for the treatment of these diseases.[2] Drug resistance has further compounded the situation.[3] This has necessitated the continued search for new chemotherapeutic agents.

The benzodiazepine derivative meclonazepam (3-methyl clonazepam) (**1**), showed a marked degree of therapeutic and prophylactic activity against all stages of schistosomiasis caused by *Schistosoma mansoni* and *S. haematobium*.[4] This drug appears to act by binding to a low affinity benzodiazepine receptor on the epidermis of the schistosome, leading to an increase in the influx of extracellular calcium and subsequent spastic paralysis of the parasite's musculature. These findings are relevant both to the considerable clinical significance of meclonazepam as well as the potential usefulness of this drug in the treatment of various other tropical diseases. One strategy to provide new drugs is the modification of existing ones to restore or enhance their activity against resistant strains of parasites or to remove unacceptable side effects such as the sedative properties of meclonazepam. In the field of tropical medicine, the development of many conventional drugs can be traced to dye precursors. This is due to the efficacy of dye chromophores against drug resistant organisms.[5,6] In addition, the dye chromophore would facilitate investigations into the location of the drug in the target organism.This prompted us to synthesize some meclonazepam dyes reported in this paper. In addition other chemical modifications were carried out to produce non-dye analogues for comparison purposes.



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The dye analogues were synthesized from meclonazepam by variation at the 7-position of the aromatic ring. The synthesis of the targeted compounds was achieved by diazotization of the amine using $NaNO_2$ and HCl at 0–4 °C followed by coupling with variously (Scheme 1) substituted phenols.[7] In addition, **MACLONAC**, **RML-1**, **A-151** were prepared with a standard combination of the reagents for the thionation[8] and

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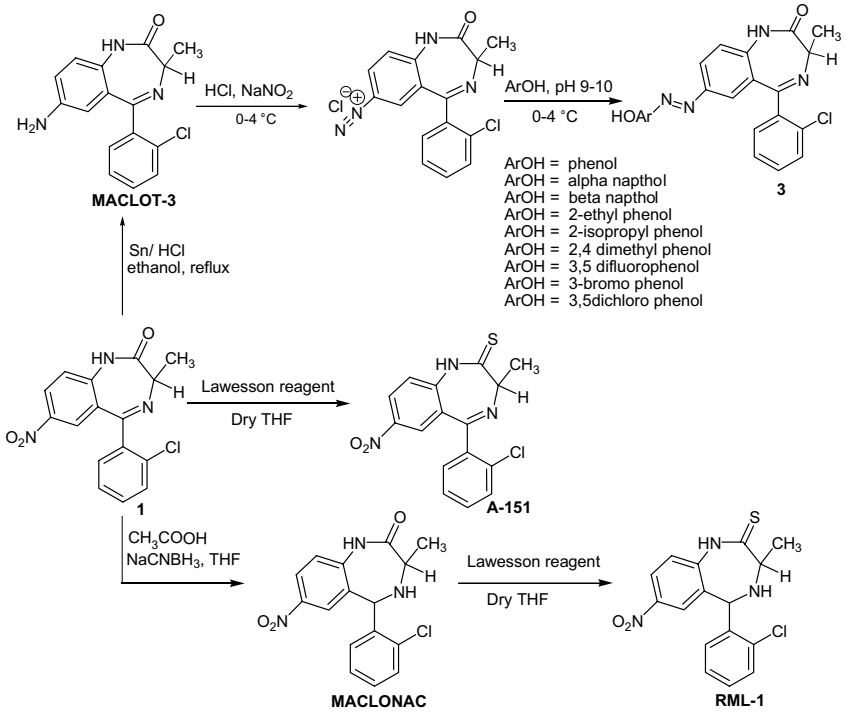

Scheme 1.

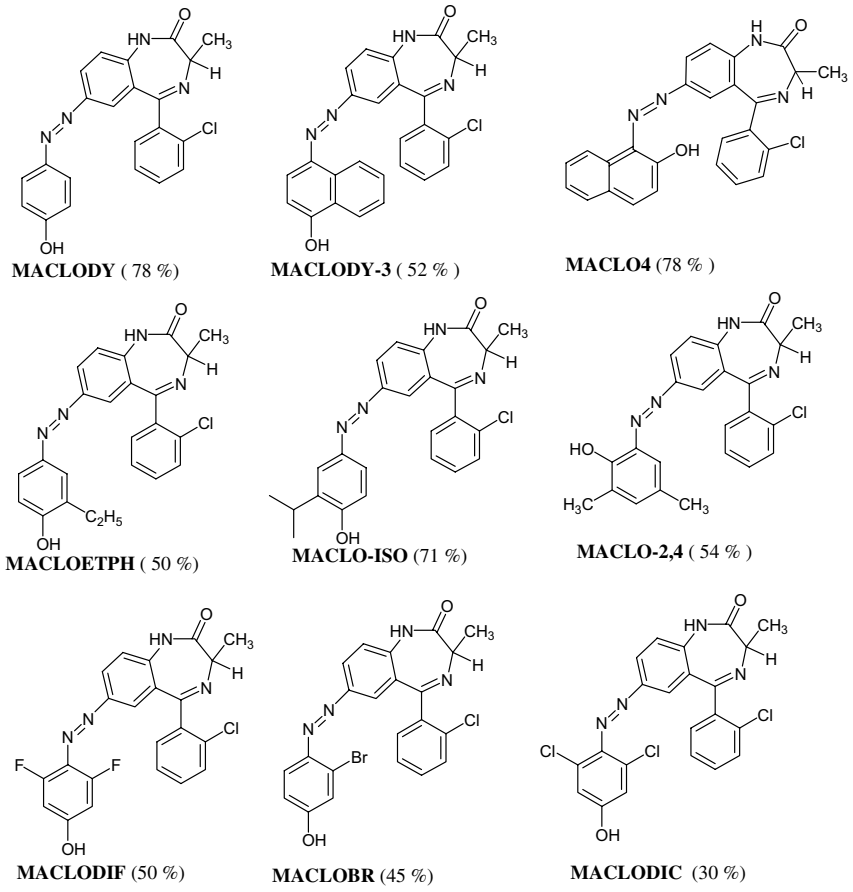

Figure 1. Diazo-derivatives of meclonazepam along with their respective yields.

reduction at positions 2 and 4 of meclonazepam. All the prepared compounds were characterized by spectroscopic and analytical means (Fig. 1).[9]

These newly synthesized analogues were tested in vitro for their activity at a concentration of 10 μg/ml against *S. mansoni*. Some compounds were effective against the parasite by killing and paralyzing the worms within 1 h of application to day 5 of the culture. The compounds produced by variation at the 2- and 4-position were highly active. Analogues **RML-1**, **MACLONAC**, **A-151** killed all worms within 1 h of application with severe damage to the teguments of the worms. This shows that substitutions can be made at 2- and 4-positions without destroying the antischistosome effects but so far such substitutions have not enhanced the IC_{50} values (Table 1). The other group of active compounds were dyes (**MACLODY-3**, **MACLODIC**, **MACLOETPH** and **MACLOISO**) produced by variation at the 7-position were slower, producing signs of damage at day 3 and death by day 5, with severe to slight tegumental damage. **MACLOBR** only killed a proportion of worms by day 5 (Table 1). Some of the diazo compounds **MACLO-4** and **MACLO-2,4**, **MACLODY** were inactive as all worms were found alive and moving. The diazo compound **MACLODIF** caused severe tegumental damage

Table 1. In vitro activity of meclonazepam analogues against *S. mansoni*

Compound	Observation[a]	IC_{50}[c] (μg/ml)
MACLODY	All worms were alive, appeared dark, granular, opaque and occasionally moved their anterior and posterior ends. One egg[b]	>10
MACLODY-3	All females and 67% males were dead by day 5 of culture (all alive on day 3). All worms looked dark, granular and opaque. Few worms showed severe tegument damage. Most worms had blebs on their surfaces. No eggs[b]	9.10
MACLO-4	All worms were alive, actively moving and stained red. Teguments of males appeared wrinkled. One egg[b]	>10
MACLOETPH	All worms were dead by day 5 of culture (all alive on day 3). All were dark, granular, opaque and slender-shaped. Few worms showed severe tegument damage and were covered in blebs. No eggs[b]	5.79
MACLOISO	All worms were dead, dark, granular and opaque by day 5 (90% dead on day 3). All showed severe tegument damage and teguments were covered in blebs. No eggs[b]	5.79
MACLO-2,4	All worms were alive, very slow in movement and were stained yellow. One egg[b]	>10
MACLODIF	All worms were dead by day 5 of culture (90% dead on day 3). All looked dark, granular, opaque, showed severe tegument damage and teguments covered in blebs. No eggs[b]	3.80
MACLOBR	Fifty percent of worms killed by day 5 of culture. Dead worms showed severe tegument damage with few blebs appearing on their teguments. Live worms occasionally moved their anterior and posterior ends. All worms looked dark, granular and opaque. No eggs[b]	10
MACLODIC	All worms were dead by day 5 of culture. All looked dark, granular and opaque. Most worms showed severe tegument damage and their surfaces were covered in blebs. No eggs[b]	5.79
MACLOT-3	No effect on worms. Some eggs[b]	>10
MACLONAC	All worms were dead within 1 h of application. By day 5, all worms were dark, granular and opaque. Females appeared thin and curled-up. All worms showed severe tegument damage and males' teguments were covered in blebs. No eggs[b]	1.30
A-151	All worms were dead within 1 h of application. On day 5, all worms were dark, granular and opaque, showed severe tegument damage and most male teguments were covered in blebs. No eggs[b]	0.35
RML-1	Some worms were killed within 1 h of application. By day 5, drug killed all males and 50% females. Live worms occasionally moved their anterior and posterior ends. All worms were dark, granular and opaque. Females appeared slender and curled-up. All worms showed severe tegument damage. Teguments of all males and most females were covered in blebs. No eggs[b]	4.75
Meclonazepam (Ro11-3128)	Some worms were killed by 1 h. By day 5, all worms were dead. All showed severe tegument damage, and looked dark, granular and opaque. No blebs formed on the teguments of dead worms. No eggs[b]	0.37

In vitro screening was performed against adult *S. mansoni* using five male and five female worms as described in reference.[10] Drug stock solutions were prepared in 100% DMSO immediately before use.

[a] Microscopic observations of motility and morphology are the criteria used for establishing drug activity. The descriptions above refer to the effects on worms of 10 μg/ml compound.

[b] Subtle drug effects are manifest by inhibition of egg production by adult worms in the cultures.

[c] IC_{50} values were calculated using Microsoft Excelfit software [IDBS Ltd, UK] from % mortality of worms that were cultured in four serial drug dilutions (1:3) starting with 10 μg/ml (10, 3.33. 1.11, 0.37 and 0.12 μg/ml).

to females, whereas **MACLOT-3** had no effect on worms at all. The low level of activity of dye analogues and **MACLOT-3** indicate that substitution at the 7-position is not favorable as the nitro group seems crucial to antischistosomal activity.

In conclusion, the data suggest that these new meclonazepam analogues could prove more promising for the future development of antischistosomal agents by introducing substituents at the 2- and 4-positions.

Acknowledgement

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9. (a) 7-Amino-5-(2-chloro-phenyl)-3-methyl-1,3–dihydro-benzo(e)(1,4)diazepin-2-one (**MACLOT-3**). Yield 50%, mp 239–241 °C, IR: (cm^{-1}) 3384 (NH_2), 3014–2988 (aromatic). ^1H NMR (400 MHz, DMSO-d_6) δ 1.69–1.7 (3H, d, $J = 6.56$ Hz, CH_3), 3.58–3.59 (2H, broad, NH, D_2O exchangeable), 3.79–3.81 (1H, q, $J = 6.57$ Hz, 1H), 6.30–6.31 (1H, d, $J = 2.61$ Hz, ArH); 6.75–6.78 (1H, dd, $J = 2.64$, 8.54 Hz, ArH); 6.89–6.90 (1H, d, $J = 8.54$ Hz, ArH); 7.29–7.31 (3H, m, ArH); 7.44–7.45 (1H, m, ArH); 8.21 (NH, D_2O, exchangeable). ^{13}C NMR: (100.6 MHz, DMSO-d_6) 16.2, 59.3, 114.1, 118.3, 122.1, 126.9, 128.5, 129.3, 129.9, 131.7, 133.1, 138.3, 143.1, 168.2, 171.9. Anal. required for $C_{16}H_{14}ClN_3O$ C, 64.11 H, 4.71 N, 14.02. Found: C, 63.04 H, 4.63 N, 13.55. FAB MS 300 $(M+1)^+$; (b) 4*E*-5-(2-Chlorophenyl)-7-(4-hydro-3-isopropylphenyl diazenyl)-3-methyl-1*H*-benzo(e)(1,4)diazepin-2(3*H*)-one (**MACLO-ISO**). Yield 71%, mp 261 °C, IR: (cm^{-1}) 3471 (OH), 3298 (NH), 3043–2870 (aromatic), 1660 (C=O), 1592 (azo). ^1H NMR (400 MHz, DMSO-d_6) δ 1.16–1.17 (6H, d, $J = 6.9$ Hz 1, 2× CH_3), 1.53–1.55 (3H, d, $J = 6.4$ Hz, CH_3), 3.20–3.25 (1H, m, $J = 6.89$ Hz, CH); 3.78–3.82 (1H, q, $J = 6.36$, CH); 6.89–6.92 (1H, d, $J = 8.57$ Hz, ArH); 7.34–7.36 (1H, d, $J = 8.75$, ArH); 7.38–7.39 (1H, d, $J = 2.14$, ArH_d); 7.45–7.49 (3H, m, ArH), 7.51–7.53 (dd, J2.42, 8.52, 1H, ArH); 7.57–7.58 (1H, m, ArH); 7.62–7.59 (1H, $J = 2.37$, ArH); 7.94–7.96 (1H, d, $J = 2.16$, 8.71 Hz, ArH); 10.2 (NH, D_2O, exchangeable); 11.0 (1H, D_2O, exchangeable OH). ^{13}C NMR: (100.6 MHz, DMSO-d_6) 17.7, 22.8, 22.9, 27.2, 59.5, 116.1, 121.6, 122.7, 123., 124.1, 125.1, 128.0, 128.4, 130.4, 131.7, 132.1, 132.6, 135.9, 139.1, 140.7, 145.5, 147.5, 159.1, 167.5, 171.1. Anal. required for $C_{25}H_{23}ClN_4O_2$ C, 67.18 H, 5.19 N, 12.54. Found: C, 67.58 H, 5.03 N, 12.01. FAB MS 447 $(M+1)^+$; (c) 5-(2-Chlorophenyl)-3-methyl-7-nitro-4,5-dihydro-1*H*-benzo(e)(1,4)diazepin-2(3*H*)-one (**MACLONAC**). Yield 90 %, mp 110–111 °C, IR: (cm^{-1}) 3688 (NH), 1692 (CN). Diasteriomeric mixture ^1H NMR (400 MHz, $CDCl_3$) δ 1.34–1.36 (3H, d, $J = 6.54$, CH_3), 1.45–1.47 (3H, d, $J = 6.93$ Hz, CH_3), 3.44–3.44 (1H, q, $J = 6.54$ Hz, 1H), 3.93–3.99 (1H, q, $J = 6.92$ Hz, 1H), 5.64 (1H, s, CH), 5.72 (1H, s, CH), 7.08–7.06 (1H, $J = 8.79$, ArH), 7.13–7.15 (1H, d, $J = 8.69$, ArH), 7.28–7.51 (9H, m, ArH), 7.80–7.82 (1H, m, ArH); 8.02–8.04 (1H, dd, $J = 2.53$, 8.76 Hz, ArH); 8.12–8.14 (1H, dd, $J = 2.53$, 8.67, ArH); 8.51 (NH, D_2O, exchangeable), 8.71 (NH, D_2O, exchangeable). presup13C NMR: (100.6 MHz, $CDCl_3$) 16.3, 18.8, 52.3, 57.1, 58.4, 61.2, 110.8, 111.1, 120.8, 121.0, 123.7, 124.1, 124.2, 124.6, 127.3, 127.7, 129.6, 129.7, 129.9, 130.0, 130.1, 134.2, 134.2, 138.4, 138.6, 142.2, 143.2, 168.0, 168.1. Anal. required for $C_{16}H_{14}ClN_3O_3$ C, 57.93 H, 4.25 N, 12.67. Found: C, 56.65 H, 4.15 N, 11.97. FAB MS 332 $(M+1)^+$; (d) (*E*)-5-(2-Chlorophenyl)-3-methyl-7-nitro-1*H*-benzo(e)[1,4]diazepine-2(3*H*)-thione (**A-151**). Yield 10%, mp 244 °C, IR: $(cm)^{-1}$ 3400 (NH), 3051–3026 (aromatic). ^1H NMR (400 MHz, $CDCl_3$) δ 1.89–1.91 (3H, d, $J = 6.33$ Hz, CH_3), 4.0–4.07 (1H, q, $J = 6.3$, 1H), 7.24–7.27 (1H, d, $J = 8.87$, ArH), 7.34–7.44 (3H, m, ArH), 7.57–7.61 (1H, m, ArH), 7.97–7.98 (1H, $d = 2.51$, $J =$ ArH), 8.31–8.35 (1H, dd, $J = 2.53$, 8.86, ArH), 9.89 (NH, D_2O, exchangeable). ^{13}C NMR: (100.6 MHz, $CDCl_3$) 20.6, 62.8, 111.2, 118.9, 121.3, 125.7, 126.6, 127.3, 127.4, 127.7, 130.4, 131.4, 143.2, 152.1, 170.5. Anal. required for $C_{16}H_{12}ClN_3O_2S$ C, 55.57 H, 3.50 N, 12.15 S, 9.27. Found: C, 53.04 H, 3.33 N, 12.07 S, 7.46. FAB MS 346 $(M+1)^+$.
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