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THE SYNTHESIS AND BIOLOGICAL ACTIVITY OF SOME KNOWN AND PUTATIVE METABOLITES OF THE ATYPICAL ANTIPSYCHOTIC AGENT OLANZAPINE (LY170053)

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Abstract: 4'-N-desmethyl olanzapine (**2**), olanzapine 4'-N-oxide (**3**) and 2-hydroxymethyl olanzapine (**5**), have been prepared and their pharmacology compared to that of the parent compound olanzapine (**1**). The 4'-N-quaternary glucuronide (**8**) has also been prepared.

Olanzapine (LY170053) (2-methyl-4-(4-methyl-1-piperazinyl)-10H-thieno[2,3-b][1,5]benzo diazepine) (**1**) is a new compound with a profile of activity in *in vitro* binding assays similar to that of the atypical antipsychotic agent clozapine[1]. Extensive *in vivo* studies of (**1**) have confirmed its potential as a novel antipsychotic agent[2,3]. Studies involving olanzapine in a number of animal species, and in man, have demonstrated the existence of several metabolic products[4,5]. The major metabolic products formed vary considerably from species to species but the same basic pathways are generally observed. Phase I metabolism involves N-demethylation and oxidation of the piperazine ring to give compounds (**2**) and (**3**), 7-hydroxylation of the fused benzene ring to give (**4**) and oxidation of the thiophene 2-methyl substituent to give (**5**) and (**7**). The aldehyde (**6**) has not been observed. Extensive phase II metabolism producing both 4'-N-glucuronide (**8**) and 10-N-glucuronide (**9**) derivatives is also observed as outlined in Figure 1.

In this paper we report the synthesis of a number of these structures related to olanzapine. These new structures have been used to aid confirmation of the identity of some of these putative metabolites. In man the major metabolite is the 10-N-glucuronide (**9**). Other minor metabolites are N-desmethyl olanzapine (**2**), olanzapine 4'-N-oxide (**3**), 2-hydroxymethyl olanzapine (**5**), and the 4'-N-quaternary glucuronide (**8**)[5]. Material containing approximately 60% of the 4'-N-quaternary glucuronide (**8**) was prepared using the procedure of Hawes *et. al*[6]. This material was sufficient to confirm the identity of material of biological origin but was of limited use for meaningful pharmacological evaluation. It was not possible to prepare the 10-N-glucuronide (**9**) by conventional chemical means. The preparation of this material using human liver slices *in-vitro* will be reported elsewhere[7]. The pharmacology of the remaining metabolites is reported.

Chemistry[8]

A number of synthetic methods have been used for the preparation of olanzapine (**1**) and related thieno[2,3-b][1,5]benzodiazepines[9]. The material for the studies reported here was prepared from the primary amidine hydrochloride (**10**) according to Scheme 1. The N-desmethyl derivative

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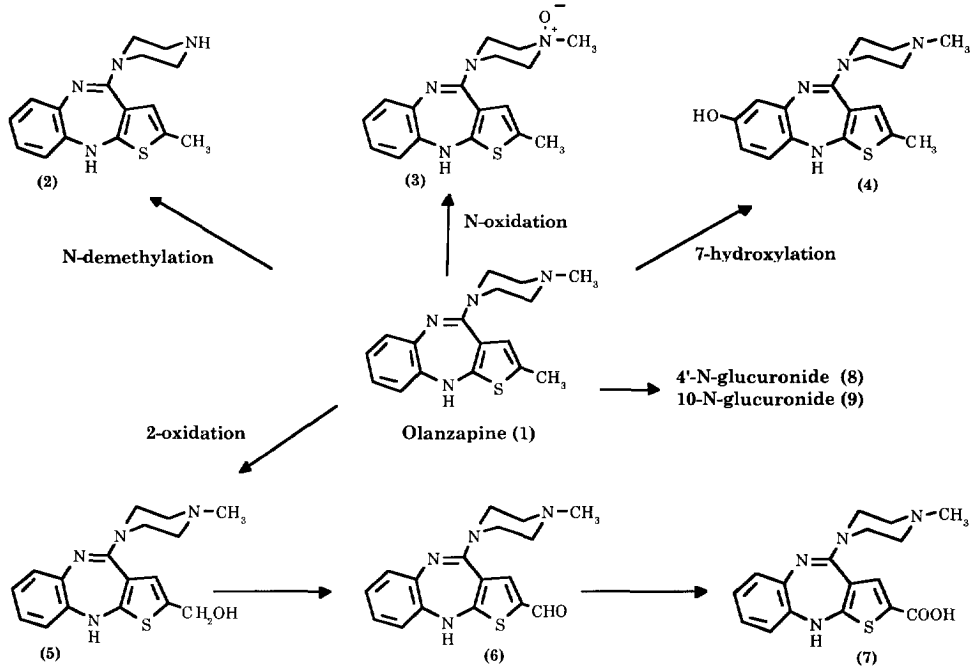
(2) was also synthesised by condensation of the primary amidine hydrochloride (**10**) with anhydrous piperazine. These reactions were best carried out in a 4:1 mixture of toluene and DMSO at reflux. Synthesis of the 4'-N-oxide (**3**) was achieved directly from olanzapine (**1**) by oxidation with *m*-chloroperbenzoic acid in methylene chloride (Scheme 1).

Figure 1. Possible transformations

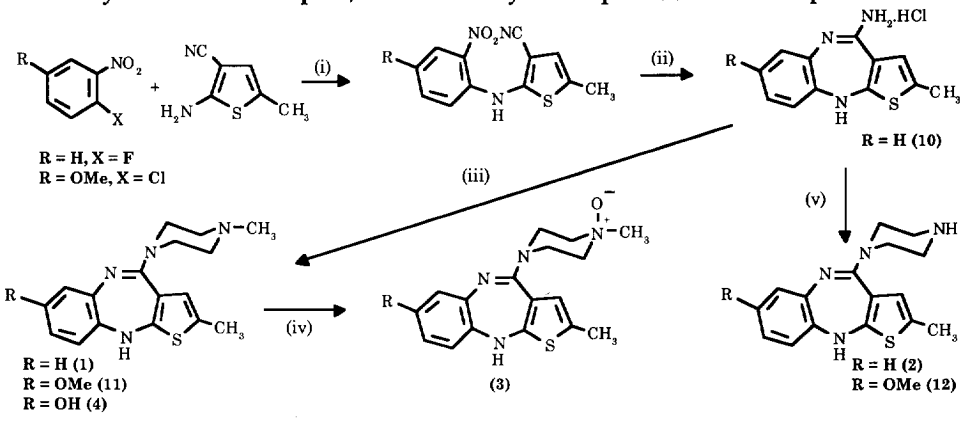


Production of the 7-hydroxy derivative (**4**) was possible by synthesis of the intermediate 7-methoxy derivative (**11**) (Scheme 1) using commercially available 4-chloro-3-nitroanisole as starting material. The condensation reaction with 2-amino-5-methylthiophene-3-carbonitrile was achieved in only poor yield using lithium hydroxide as base in dimethylsulfoxide. Other methods to achieve this condensation failed. The 7-hydroxy derivative (**4**) was a very unstable compound in solution. Biological samples containing material of metabolic origin were therefore derivatised with diazomethane and compared with the methoxy derivative (**11**). The 4'-N-desmethyl-7-methoxy derivative (**12**) was similarly prepared.

The unsubstituted nitro nitrile (**13**) underwent Vilsmeier-Hack formylation using $POCl_3$/DMF to give the key intermediate aldehyde (**14**) (Scheme 2). The aldehyde (**14**) was oxidised using Jones reagent in acetone to give modest yields of the acid (**15**). Diazomethane in methylene chloride gave the ester (**16**) in quantitative yield. This ester underwent reductive cyclisation to the primary amidine ester (**17**) using $SnCl_2$ in aqueous ethanolic HCl in high yield. Displacement of the primary amidine with N-methyl piperazine resulted in a 60% yield of the required ester (**18**). Confirmation that the acid (**7**) was a metabolite in some animal species but not in man, was achieved by treatment of biological samples with diazomethane and comparison with

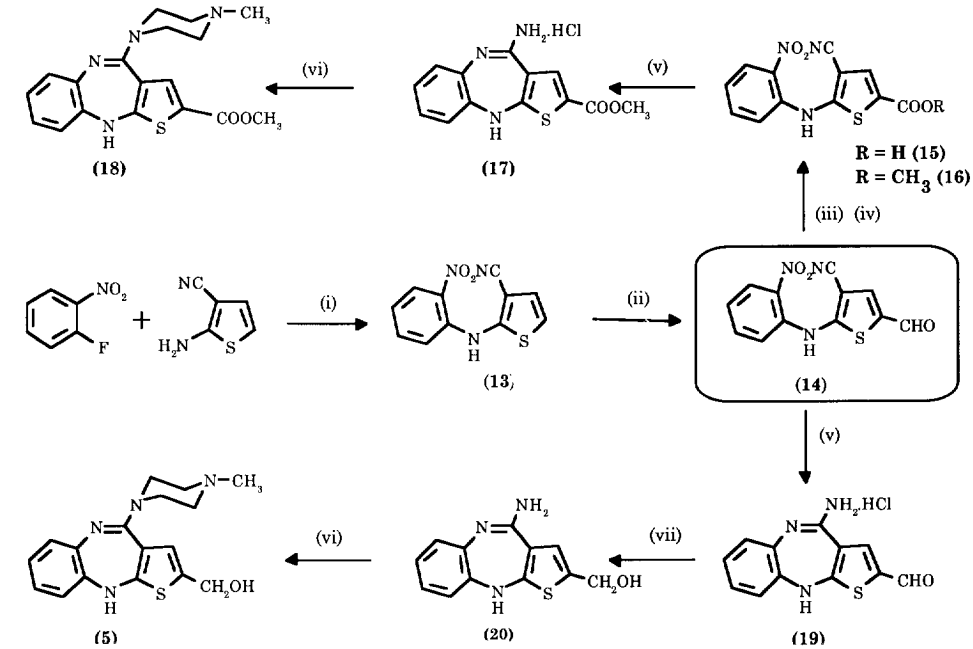
ester (**18**). The key intermediate aldehyde (**14**) could be reductively cyclised in high yield to the primary amidine aldehyde (**19**). All attempts to achieve displacement with N-methyl piperazine failed. The primary amidine aldehyde (**19**) was a moderately unstable compound which could not

Scheme 1. Synthesis of olanzapine, 4'-N-desmethylolanzapine (2) and olanzapine 4'-N-oxide (3)



Reagents: (i) LiOH/DMSO; (ii) $SnCl_2$/EtOH; (iii) NMP/Toluene/DMSO; (iv) *m*-CPBA/CH_2Cl_2 (v) Anhydrous piperazine/Toluene/DMSO.

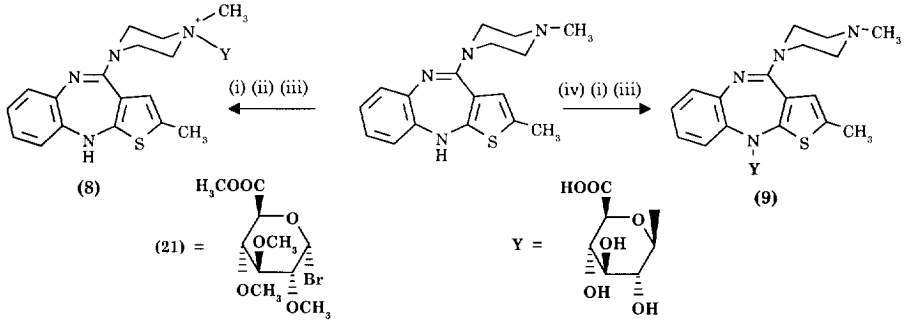
Scheme 2 Reactions of thiophene-2-carboxaldehyde derivative.



Reagents: (i) LiOH/DMSO; (ii) $POCl_3$/DMF; (iii) CrO_3/H_2SO_4/Acetone; (iv) CH_2N_2; (v) $SnCl_2$/EtOH/HCl; (vi) NMP/Toluene/DMSO; (vii) $NaBH_4$/EtOH.

be chromatographed or crystallised to high purity. Reduction of the crude product with $NaBH_4$ in ethanol, however, gave the primary amidine alcohol (**20**) which was a stable material and could be obtained analytically pure. Displacement of this primary amidine under the usual conditions resulted in the formation of the putative metabolite (**5**) in high yield. Attempts were made to prepare both the 4'-N glucuronide (**8**) and 10-N glucuronide (**9**) derivatives (Scheme 3). We have previously shown that it is possible to achieve alkylation of the 10-position of the thieno[2,3-b][1,5]benzodiazepine nucleus with secondary halides using sodium hydride in dimethylformamide, primary halides working well with *n*-BuLi in the presence of tetramethylethylene diamine[10].

Scheme 3. Attempted synthesis of 4'-N (8) and 10-N- glucuronide (9) derivatives.



Reagents; (i) **21**; (ii) Amberlite XAD-2; (iii) HCl (iv) NaH/THF or *n*-BuLi/THF/TMEDA.

Attempts to achieve alkylation using methyl-(tri-O-acetyl-α-D-glucopyranosyl bromide)-uronate (**21**)[11] under these conditions failed to yield any of the desired product after hydrolysis. Direct reaction of (**1**) with (**21**) under the conditions of Hawes[6] produced the expected quaternary 4'-N-glucuronide (**9**) after ion-exchange chromatography but the yield was extremely low (1-2%) and it was contaminated with a second quaternary species of unknown structure. Capillary zone electrophoresis (CZE) demonstrated that the product contained approximately 40% of the required material and 60% of the unknown impurity. No separation could be achieved by any chromatographic techniques tried. Preparative electrophoresis also failed. Some enrichment of the required material was achieved by ethanol precipitation from an aqueous solution. This resulted in a sample containing 60% of the quaternary 4'-N-glucuronide (**8**). This material was used to confirm that (**8**) was a minor metabolite in man[4]

In vitro pharmacology

The 10-N-glucuronide derivative (**9**) was devoid of activity in any of the binding assays used (Ki >10μM). The *in vitro* binding affinities of the three minor human metabolites of olanzapine (**1**), the 4'-N-desmethyl (**2**), the 4'-N-oxide (**3**) and the 2-hydroxymethyl derivative (**5**) were determined compared to parent for a range of receptor subtypes. The data are reported in Table 1 expressed as Ki (nM). Of these compounds both the 4'-N-desmethyl derivative (**2**) and the 2-hydroxymethyl derivative (**5**) exhibited activity and receptor binding profiles similar to those of olanzapine. The 4'-N-oxide (**3**) was essentially devoid of activity. The intermediate 7-methoxy derivative (**11**) and

the ester (**18**) were tested for affinity at dopamine D_2 receptors and found to be only weakly active (Ki 250 nM and 100 nM respectively).

Table 1. Radioligand binding (Ki nM) of olanzapine (1), N-desmethyl-olanzapine (2), the 4'-N-oxide of olanzapine (3) and 2-hydroxymethyl-olanzapine (5) to neuronal receptors[1].

Receptor	Olanzapine (1)	N-desmethyl olanzapine (2)	4'-N-oxide (3)	2-hydroxymethyl olanzapine (5)
Dopamine D_1	119±20	203±7	395±285	66±14
Dopamine D_2	20±8	9±0.2	119±8	22±0.7
$5HT_2$	10	23±5	112±3	18±2
Muscarinic	68±4	333±64	662±169	501±119
Histamine H_1	7±0.3	22±0.2	87±0.5	6±2
α_1-adrenergic	15±3	80±6	266±2	12±1
α_2-adrenergic	263±12	1000±189	>10000	783±44
β-adrenergic	>10000	>10000	>10000	>10000
$GABA_A$	>10000	>10000	>10000	>10000
Benzodiazepine	>10000	>10000	>10000	>1000

***In vivo* pharmacology**

The *in vivo* activity of olanzapine was compared to that of the three minor metabolites on apomorphine-induced climbing behaviour in mice[12] and in their ability to block conditioned avoidance behaviour in rats[13,14]. Olanzapine (ED_{min} 0.625 mg/kg s.c., 0.1 mg/kg i.v. or 2.5 mg/kg p.o.) significantly reduced apomorphine-induced climbing behaviour in mice (Table 2). The 4'-N-desmethyl (**2**) and 2-hydroxymethyl (**5**) metabolites failed to antagonise climbing behaviour at doses less than 20 mg/kg. The 4'-N-oxide of olanzapine (**3**) was the most active agent, blocking apomorphine-induced climbing behaviour with a minimum effective dose of 12.5 mg/kg sc, compared to an EDmin of 0.625 mg/kg sc for olanzapine.

Table 2. In vivo pharmacology

Compound	Apomorphine Climbing[15] ED_{min} (mg/kg)			CAR[16] ED_{min}(mg/kg)	
	sc	iv	po	iv	po
Olanzapine (**1**)	0.625	1.0	2.5	0.25	2.5
N-desmethyl olanzapine (**2**)	50	>10	NT	>4	>25
4'-N-oxide (**3**)	12.5	>10	NT	>4	>25
2-hydroxymethyl olanzapine (**5**)	NT	NT	20	NT	NT

Olanzapine (**1**) (0.125 - 1 mg/kg iv and 2.5 - 10 mg/kg po) produced a dose-related reduction in avoidance responding in rats with an ED_{min} of 0.25mg/kg iv and 2.5 mg/kg po respectively. 4'-N-desmethyl olanzapine (**2**) (25 mg/kg po or 1 and 4 mg/kg iv), and the 4'-N-oxide (**3**) (25 mg/kg po or 4 mg/kg iv) had no effect on this response[14] (Table 2).

Discussion

These studies confirm previous reports demonstrating that olanzapine (**1**) antagonised apomorphine-induced climbing behaviour and blocked a conditioned avoidance response in rats[2]. Based on these studies it would be predicted that it will be an effective antipsychotic agent.

All the metabolites tested were significantly less active *in vivo* than the parent compound. The 4'-N-oxide of olanzapine (**3**) was the most active agent tested, blocking apomorphine-induced climbing behaviour with an EDmin of 12.5 mg/kg sc, compared to an EDmin of 0.625 mg/kg for olanzapine. Metabolite (**3**) administered by the iv route failed to antagonise the climbing response or block conditioned avoidance behaviour. The lack of activity of 4'-N-desmethyl-olanzapine (**2**) and 2-hydroxymethyl-olanzapine (**5**) demonstrates that although these agents have affinity for D_2 receptors similar to that of olanzapine (**1**), this does not translate into functional activity *in vivo*.

In conclusion, these data demonstrate that all metabolites are significantly less active than olanzapine. It is therefore unlikely that the activity of these agents contributes to the overall pharmacological profile of the parent compound.

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15. Groups of five animals per dose level.
16. Groups of eight animals per dose level.

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