



GEORGIA INSTITUTE OF TECHNOLOGY  
OFFICE OF CONTRACT ADMINISTRATION

NOTICE OF PROJECT CLOSEOUT

Closeout Notice Date 03/19/91

Project No. G-33-667 \_\_\_\_\_ Center No. 10/24-6-R6834-1A0\_  
Project Director ZALKOW L H \_\_\_\_\_ School/Lab CHEMISTRY \_\_\_\_\_  
Sponsor DHHS/PHS/ADAMHA/ALCOHOL, DRUG ABUSE & MENTAL \_\_\_\_\_  
Contract/Grant No. 1 R01 DA06305-01 \_\_\_\_\_ Contract Entity GTRC  
Prime Contract No. \_\_\_\_\_  
Title IRREVERSIBLE ANTAGONISTS OF COCAINE AND OTHER STIMULANTS \_\_\_\_\_  
Effective Completion Date 901130 (Performance) 910228 (Reports)

Closeout Actions Required:	Y/N	Date Submitted
Final Invoice or Copy of Final Invoice	Y	_____
Final Report of Inventions and/or Subcontracts	N	_____
Government Property Inventory & Related Certificate	N	_____
Classified Material Certificate	N	_____
Release and Assignment	N	_____
Other _____	N	_____

Comments CONTINUED BY G-33-608; FINANCIAL STATUS REPORT IS REQUIRED. \_\_\_\_\_

Subproject Under Main Project No. \_\_\_\_\_

Continues Project No. \_\_\_\_\_

Distribution Required:

Project Director	Y
Administrative Network Representative	Y
GTRI Accounting/Grants and Contracts	Y
Procurement/Supply Services	Y
Research Property Management	Y
Research Security Services	N
Reports Coordinator (OCA)	Y
GTRC	Y
Project File	Y
Other _____	N
_____	N

SECTION IV PROGRESS REPORT SUMMARY		GRANT NUMBER DA06305-02	
PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR Zalkow, Leon H.		PERIOD COVERED BY THIS REPORT	
APPLICANT ORGANIZATION Georgia Institute of Technology		FROM 9/30/89	THROUGH 11/30/90
TITLE OF PROJECT (Repeat title shown in item 1 on first page) Irreversible Antagonists of Cocaine and Other Stimulants (SEE INSTRUCTIONS)			

## 1. SUMMARY OF PLANS FOR NEXT YEAR OF SUPPORT

No significant changes are planned over those outlined in the original proposal. Specifically we will:

### A. Chemistry

1. Complete all synthetic work related to the GBR series of aryl 1,4-dialk(en)ylpiperazines.
2. Complete all synthetic work related to the mazindol series of imidazo-isoindols.
3. Continue synthetic work on the methylphenidate series.
4. Start work on on the dopamine series or another new structure if appropriate.

### B. Pharmacology

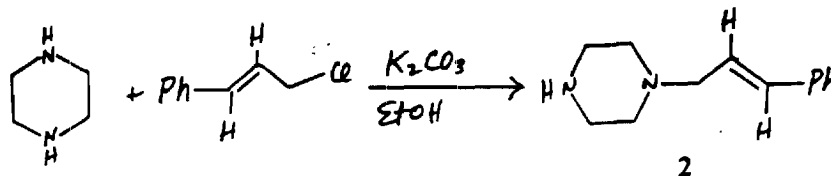
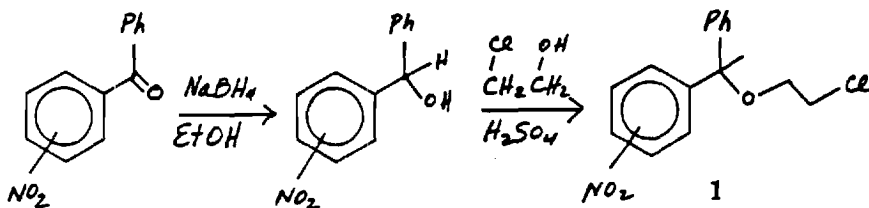
1. Complete all testing of the GBR series.
2. Start and largely complete work on the mazindol series.
3. Start work on the methylphenidate series.

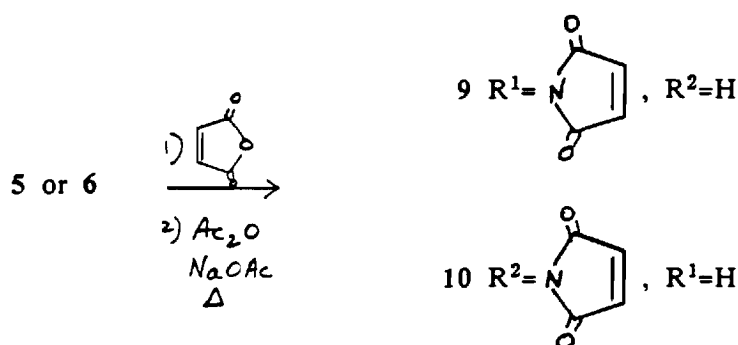
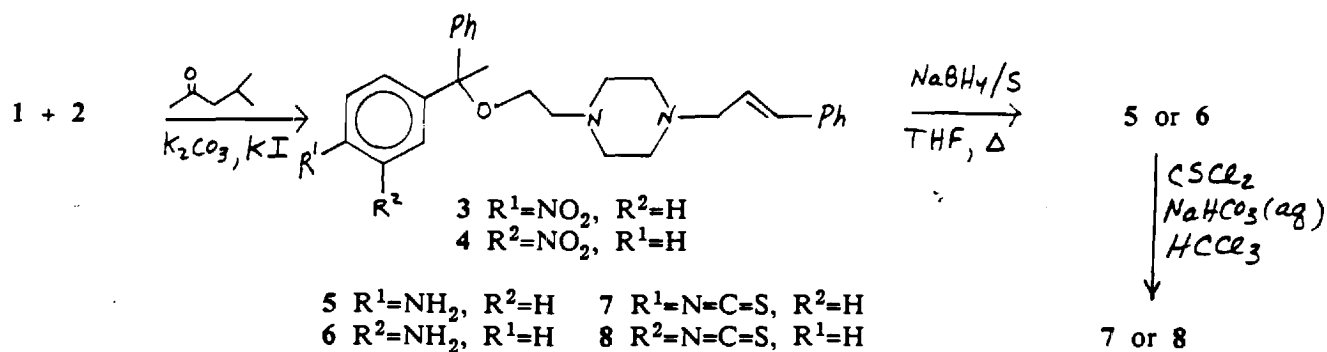
## 2. DESCRIPTION OF WORK CONDUCTED IN CURRENT YEAR (9/30/89 TO 11/30/90)

### A. Chemistry

#### 1. GBR Series

Synthetic work was initiated in this series using the methods outlined in the the literature with a number of significant modifications and additions. This work is summarized below:

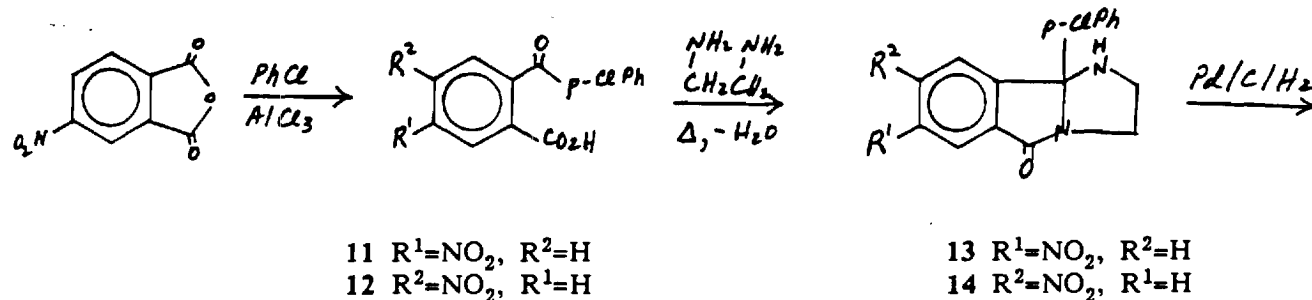


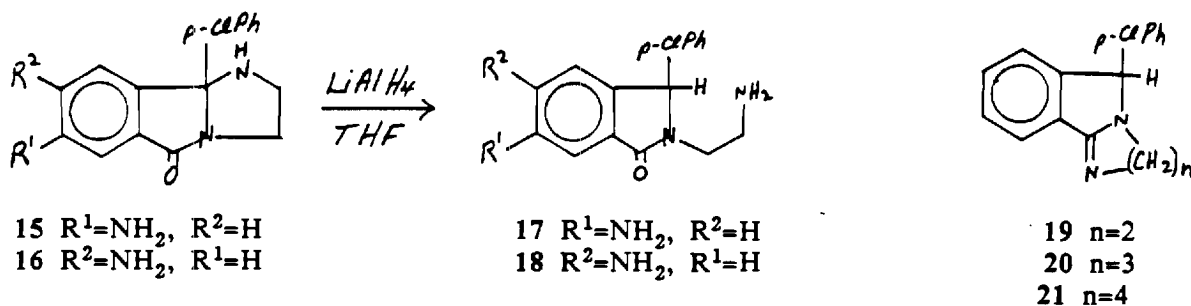


New compounds 3-10 have been synthesized, fully characterized and submitted for screening in the methylphenidate radioligand assay. These results are summarized in section B.2.

## 2. Mazindol Series

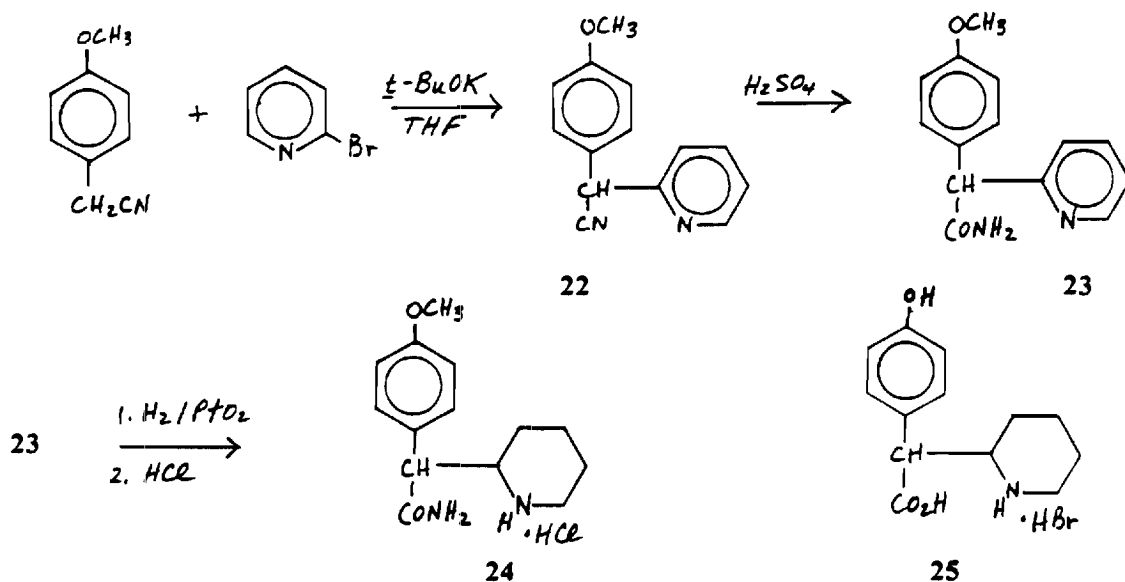
The Friedel-Crafts acylation of chlorobenzene with 4-nitrophthalic anhydride gives a 4:1 mixture of the expected 5-nitro product (11) to the 4-nitro product (12). This mixture can be separated by crystallization. Condensation of 11 or 12 with ethylene diamine gives 13 or 14 which on reduction gives 15 or 16, respectively. Lithium aluminum hydride reduction of 15 or 16 does not give the expected products, but instead gives materials formulated as 17 or 18, respectively. Alternative methods to convert these to the desired final products are being explored. We have also synthesized the unsubstituted mazindol series 19-21, which will be screened.





### 3. Methylphenidate Series

The reaction of *p*-methoxyphenylacetonitrile with 2-bromopyridine according to the literature procedure gave a difficult to separate mixture from which the desired product (22) could be separated by chromatography in very low yield. After modification of the conditions, satisfactory yields (isolated by crystallization) were obtained as shown below. Partial hydrolysis of 22 gave 23 which upon hydrogenation gave 24 as a mixture of *erythro* (predominate) and *threo* isomers. Deprotection and isomerization of 24 was achieved in excellent yield using 48% HBr yielding 25 in which the predominate *threo* isomer could be separated by crystallization. After conversion of *threo* 25 to the methyl ester (*p*-hydroxymethylphenidate) this compound will be screened before further work in undertaken.



## B. Pharmacology

### 1. Fourphit

#### a. Biochemical studies.

*In vitro* studies examining the effect of Fourphit on [ $^3H$ ]methylphenidate binding were completed. This isothiocyanate derivative of phencyclidine was found to inhibit binding to the stimulant recognition site with an  $IC_{50}$  of 7.1  $\mu M$  (Fig.1). Washout studies (conducted under conditions where reversibly bound phencyclidine was completely removed) demonstrated that inactivation of the stimulant recognition site by Fourphit was irreversible, while inactivation of the phencyclidine receptor by the compound was reversible (Fig. 2). Partial protection of the

stimulant recognition site from inactivation by Fourphit was afforded by pretreatment of striatal tissue with saturating amounts of unlabeled methylphenidate, suggesting that the acylating compound may bind covalently to the receptor directly at the site recognized by methylphenidate, rather than allosterically (Table 1). Studies examining the effects of Fourphit on the dissociation rate of [<sup>3</sup>H]methylphenidate also supported the interpretation that the inhibition caused by Fourphit was not due to an allosteric interaction of the compound with the stimulant recognition site (Table 2).

#### b. Behavioral studies.

Intravenous injection of Fourphit (20 mg/kg) had little or no effect on the activity levels of rats. On the other hand, the activity levels of rats in response to a challenge with selected stimulant agents was altered by pretreatment with Fourphit 24 hrs in advance, as compared to rats injected with vehicle alone one day prior to administration of the stimulant drugs. The effect of Fourphit on stimulant-induced locomotion was not constant, but appeared to vary with the respective stimulants. As predicted in the original proposal, Fourphit attenuated the hyperactivity induced by challenge with a low dose of cocaine (Fig. 3). Rats pretreated with Fourphit prior to challenge with methylphenidate (Ritalin), which belongs to the same class of stimulant agents as cocaine, however, showed no change (or possibly even a slight increase) in activity, compared to vehicle-pretreated control animals injected with methylphenidate (Fig. 4). Pretreatment with Fourphit significantly increased locomotor activity observed following low doses of d-amphetamine (0.8 and 1.25 mg/kg; Figs. 5 and 6, respectively), while it was without effect on the activation induced by 1.5 mg/kg of methamphetamine (Fig. 7), which belongs to the same class of stimulant drugs as amphetamine.

*In vitro* measurement of [<sup>3</sup>H]methylphenidate binding in striatal tissue dissected from the rats utilized in the behavioral studies described above revealed no consistent changes supportive of altered dopamine transport function. Future studies will examine binding of [<sup>3</sup>H]methylphenidate closer to the time of Fourphit administration, the hypothesis being that interference with uptake of dopamine occurring soon after injection of the phencyclidine derivative may lead to compensatory changes in dopamine uptake, metabolism, and/or release which differentially affect the animal's response to the various stimulant agents.

## 2. GBR Series

The parent compound (GBR-12783) and intermediate 3 containing no reactive moiety were compared with 7 and 9 with respect to their effects on [<sup>3</sup>H]methylphenidate binding. A summary of the results is shown in Table 3. All of the compounds inhibited binding to the stimulant recognition site, with the following order of potency: GBR-12783 > 3 > 7 > 9. The inhibition was marked by a Hill coefficient greater than one in all cases. Washout studies using a sodium-free solution for removing reversibly-bound compounds showed that only the isothiocyanate-substituted compound bound irreversibly to the stimulant recognition site. Future work will focus on characterization of the effect of 7 on [<sup>3</sup>H]methylphenidate binding, dopamine transport, and stimulant-induced behaviors. Initial screening of the other compounds in this series is planned for the immediate future.

## 3. NO HUMAN SUBJECTS

## 4. NO CHANGE IN THE USE OF ANIMALS (ASSURANCE #A1575)

## 5. PUBLICATIONS:

Manuscripts are in preparation concerning the effects of fourphit and the synthesis and pharmacology of the GBR compounds.

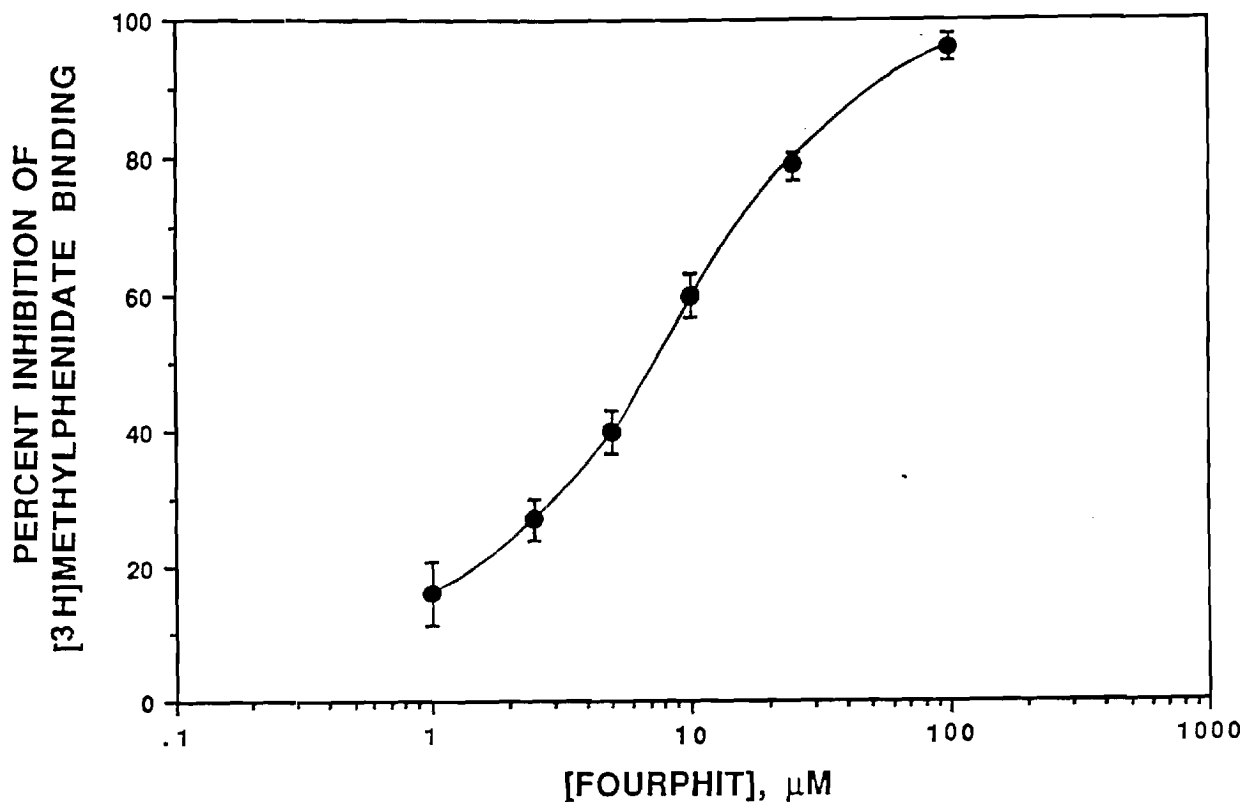
INHIBITION OF [<sup>3</sup>H]METHYLPHENIDATE  
BINDING BY FOURPHIT

Fig. 1. Inhibition of [<sup>3</sup>H]methylphenidate binding by Fourphit. Binding of [<sup>3</sup>H]methylphenidate was determined in samples containing aliquots of a P<sub>2</sub> fraction suspended in Tris assay buffer and incubated for 30 min at 0°C with 7.6 nM [<sup>3</sup>H]methylphenidate and varying concentrations of Fourphit). Data shown are means ± SEM of triplicate samples from one experiment, which was repeated twice more with similar results.

### EFFECT OF FOURPHIT ON [<sup>3</sup>H]METHYLPHENIDATE AND [<sup>3</sup>H]TCP BINDING

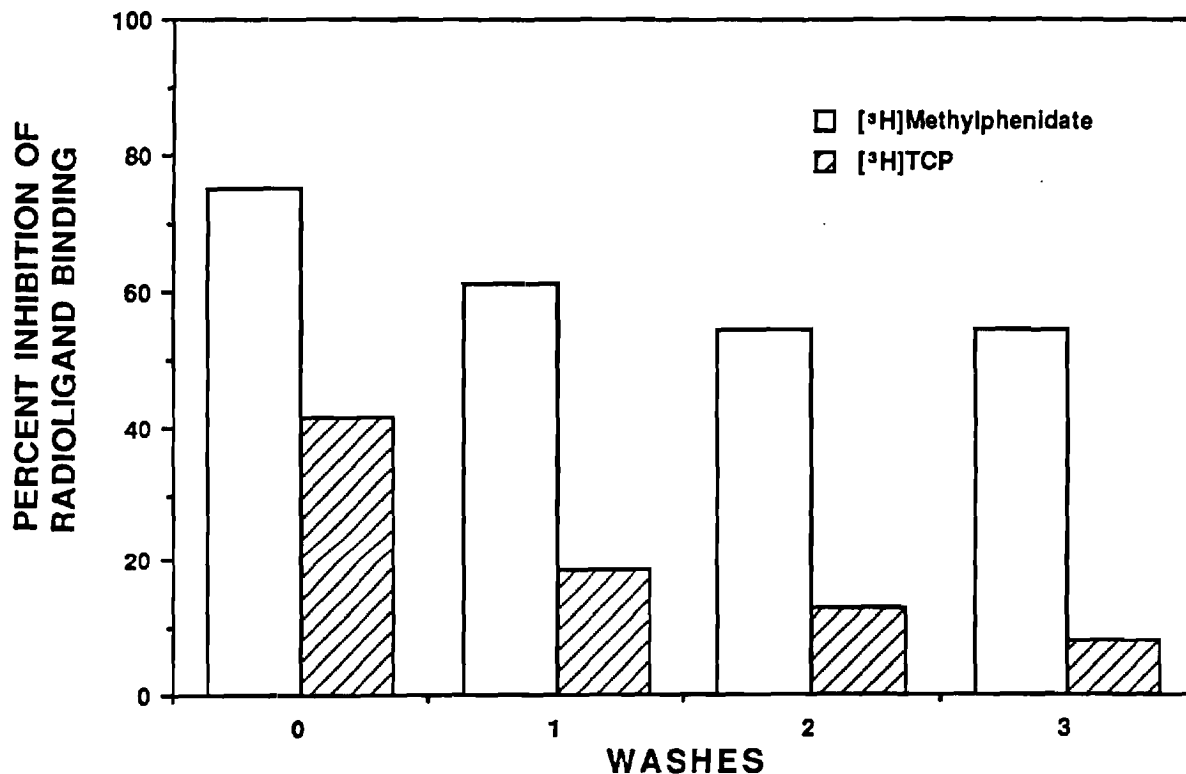


Fig. 2 . Effect of Fourphit on [<sup>3</sup>H]methylphenidate and [<sup>3</sup>H]TCP binding. In studies of [<sup>3</sup>H]methylphenidate binding (open bars), a crude synaptosomal preparation of striatal tissue was reacted with 28.6  $\mu$ M Fourphit for 20 min at 0°C. In studies of [<sup>3</sup>H]TCP binding (hatched bars), tissue was reacted with 40  $\mu$ M Fourphit for 30 min at 5 ° C. Binding in Fourphit-treated samples was compared with vehicle-treated controls before ("0 wash") and after removal of the drug-containing supernatant by repeated centrifugation and resuspension. Concentration of radioligand used to determine binding was 8.9 and 2 nM for [<sup>3</sup>H]methylphenidate and [<sup>3</sup>H]TCP, respectively. Data shown are means of triplicate samples from one experiment, which was repeated twice more with similar results. For the data illustrated, the standard error of the mean ranged from 0.5 - 2.8%.

Table 1. Protection by excess methylphenidate of the stimulant recognition site from inactivation by Fourphit

Percent inhibition of [ <sup>3</sup> H]methylphenidate binding			
Tissue status	Methylphenidate	Fourphit	Protected
Unwashed	99.6±0.3	73.0±2.7	99.9±0.3
Washed	1.5±3.7	40.8±1.8	34.9±1.0*

Inhibition of [<sup>3</sup>H]methylphenidate binding was determined before and after washing in samples (1) pretreated with 50  $\mu$ M methylphenidate but not exposed to Fourphit ("Methylphenidate"); (2) not pretreated with methylphenidate, but exposed to 28.6  $\mu$ M Fourphit ("Fourphit"); and (3) pretreated with 50  $\mu$ M methylphenidate before exposure to 28.6  $\mu$ M Fourphit ("Protected"). See Materials and Methods for experimental details. Control binding before and after washing was  $61 \pm 8$  and  $46 \pm 6 \times 10^3$  CPM's/mg protein, respectively (mean  $\pm$  SEM from three separate experiments). Percent inhibition in protected samples after washing was calculated by comparison to the washed "Methylphenidate" samples; inhibition in all other samples was calculated by comparison to untreated controls. \*Significantly different from washed "Fourphit" (one-tailed paired t-test,  $P < 0.015$ ).

TABLE 2. Effect of Fourphit on the dissociation of [<sup>3</sup>H]methylphenidate

Series	Incubated with	Dissociation initiated with	k <sub>11</sub> (min <sup>-1</sup> )	k <sub>12</sub> (min <sup>-1</sup> )
1	Vehicle	10 μM amfonelic acid	0.714 ± 0.048	- - -
	5 μM Fourphit	10 μM amfonelic acid	0.744 ± 0.036	- - -
2	Vehicle	100 μM methylphenidate	1.22 ± 0.14	0.281 ± 0.025
	Vehicle	200 μM Fourphit	1.30 ± 0.20	0.126 ± 0.045*

In the first series of experiments, a P2 fraction was incubated with 9 nM [<sup>3</sup>H]methylphenidate and either vehicle or 5 μM Fourphit for 30 min before initiating dissociation with 10 μM amfonelic acid. At the end of the 30 min incubation period (but before the addition of the amfonelic acid), specific binding of [<sup>3</sup>H]methylphenidate to the Fourphit-treated samples was 50.5 ± 5.5 % of that in the vehicle-treated samples. In the second series of experiments, samples were equilibrated with 9 nM [<sup>3</sup>H]methylphenidate for 30 min before initiating dissociation with either 100 μM methylphenidate or 200 μM Fourphit. See Materials and Methods for details. Dissociation rates were determined using the KINETICS program (Biosoft). Results shown are means ± SEM from three separate experiments; duplicate samples were run within each experiment. \*Significantly different from k<sub>12</sub> obtained using 100 μM methylphenidate to initiate dissociation (P<0.029, one-tailed paired t-test).

Figure 3  
**FOURPHIT EFFECT on COCAINE BEHAVIOR**

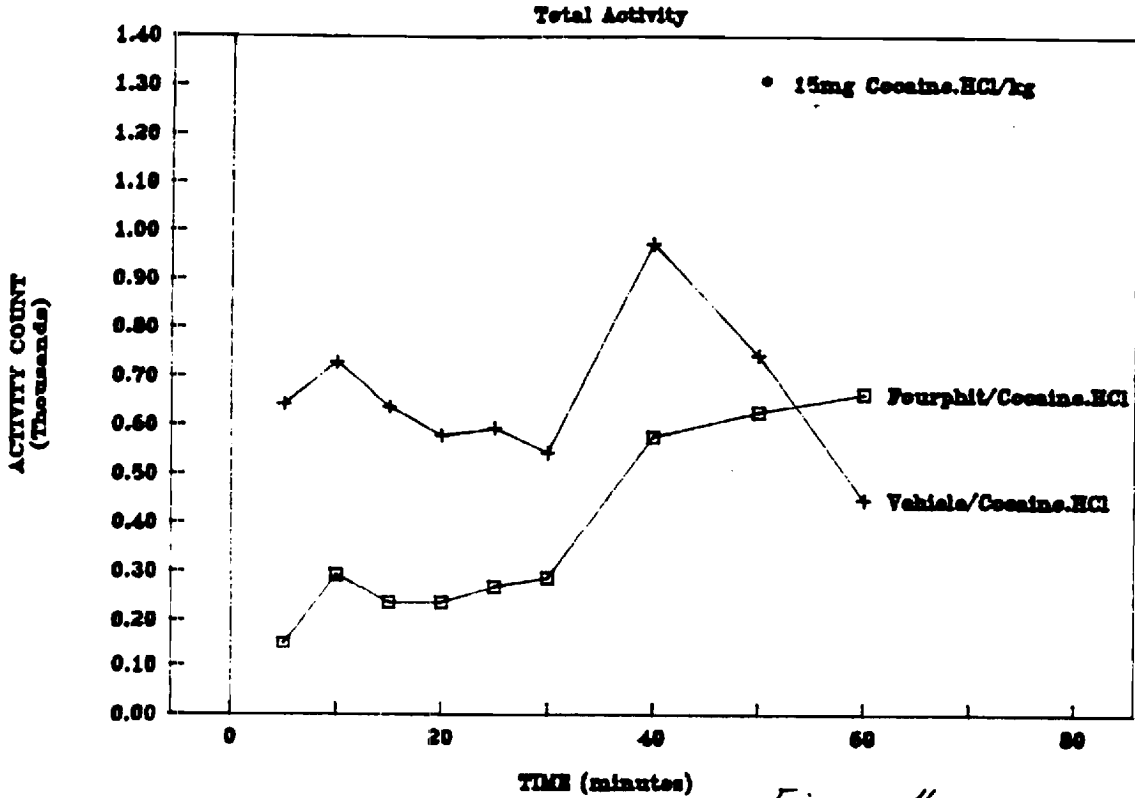


Figure 4  
**FOURPHIT EFFECT on RITALIN BEHAVIOR**

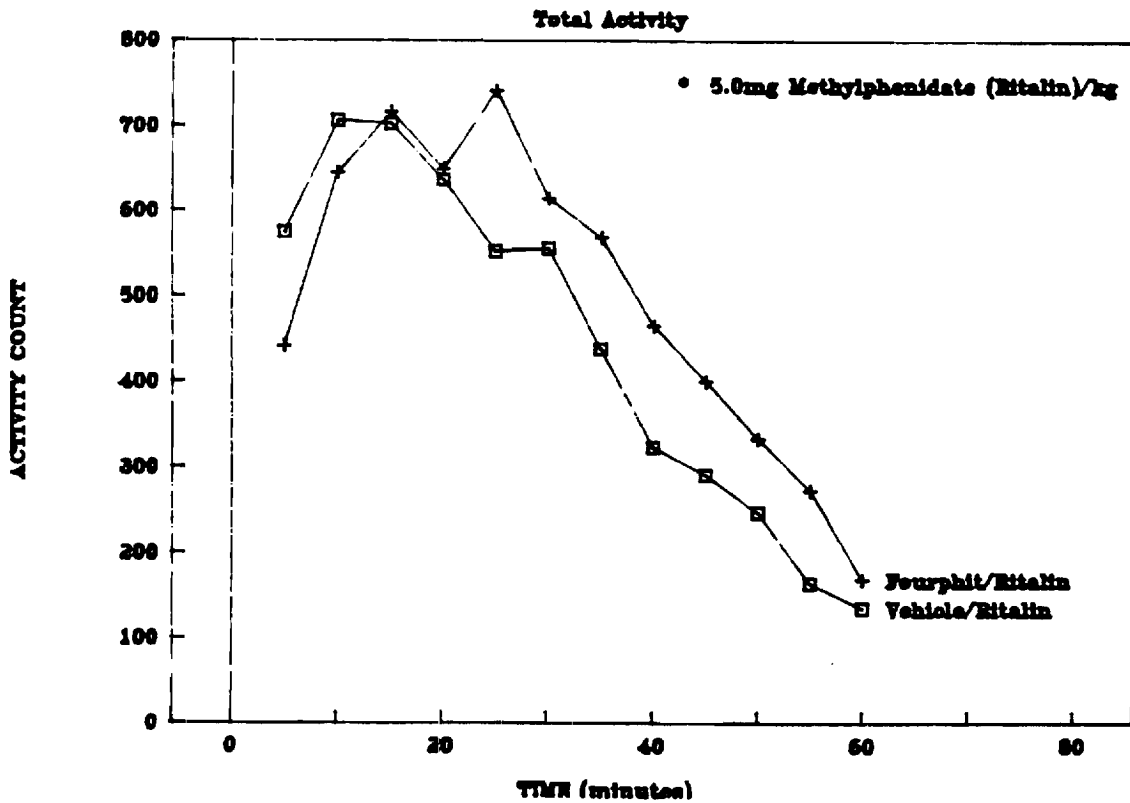


Figure 5

FOURPHIT EFFECT on AMPHETAMINE BEHAVIOR

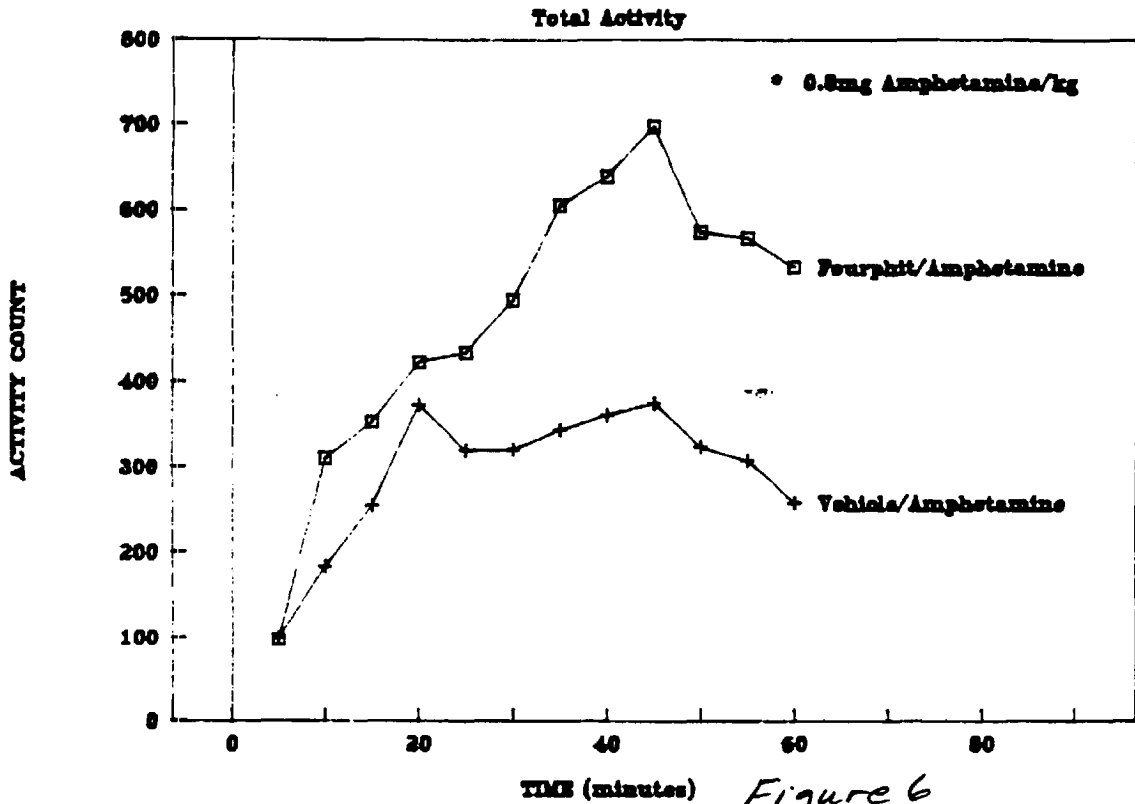


Figure 6

FOURPHIT EFFECT on AMPHETAMINE BEHAVIOR

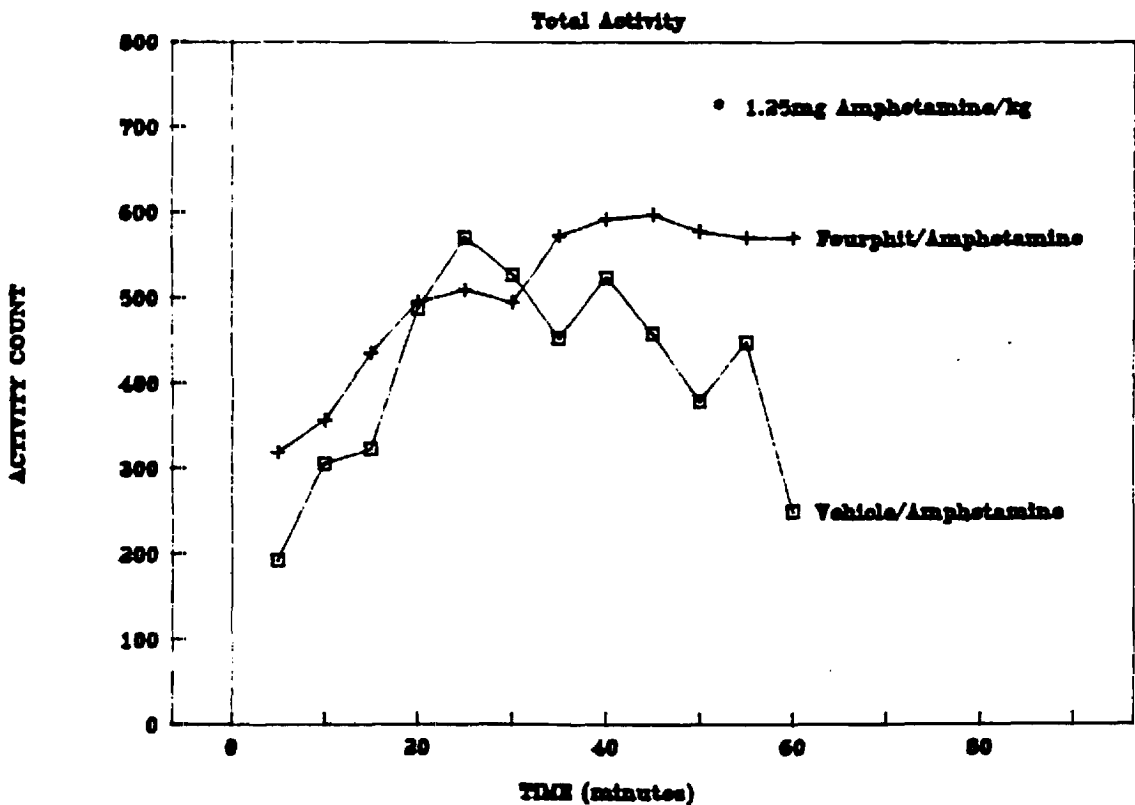
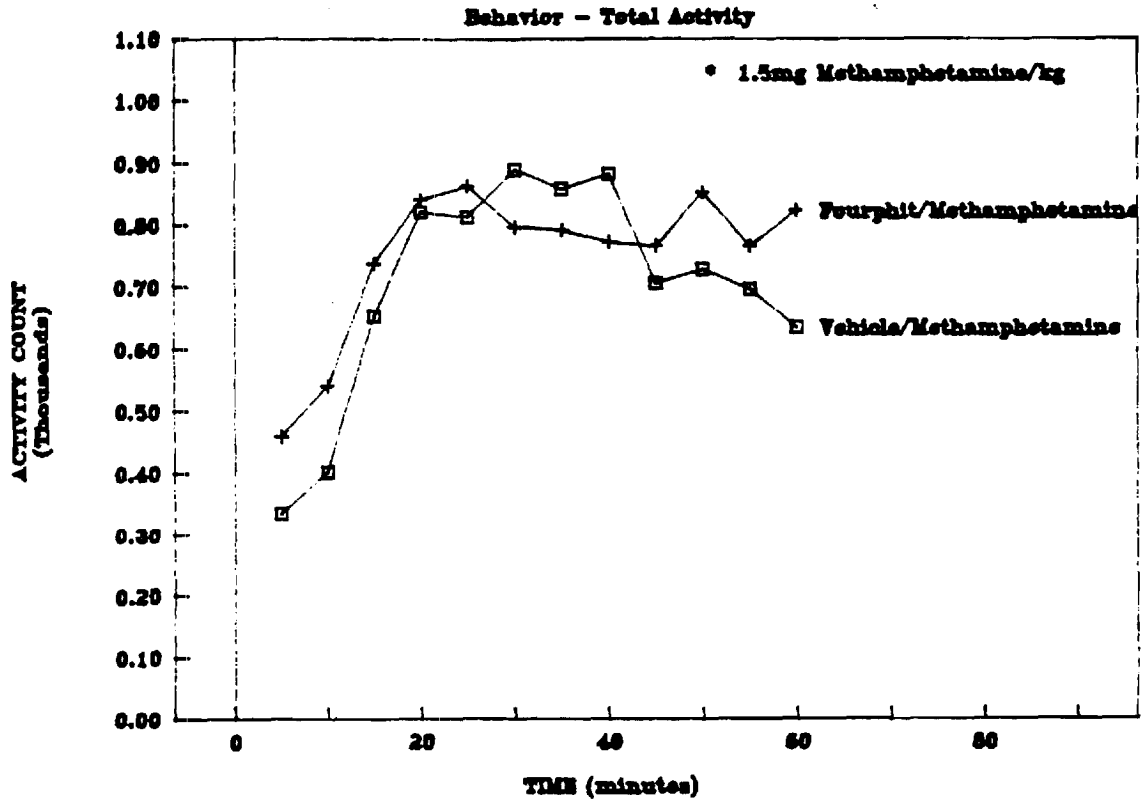


Figure 7

## FOURPHIT EFFECT ON METHAMPHETAMINE



Figs. 3 - 7 . Rats were housed individually overnight. After injection with Fourphit (20 mg/kg, i.v.) or vehicle, they were returned to their home cages. Twenty-four hours later, they were challenged (i.p.) with a stimulant agent (cocaine, methylphenidate, amphetamine, or methamphetamine) or saline vehicle, the animals were returned to their home cages, and each individual cage was placed in an animal activity monitoring apparatus where activity was measured at 5 min intervals over a period of 60 min. (The activity was quantitated by counting the number of times the IR light beams were broken as the animal moved about the cage.)

TABLE 3. Effect of GBR-12783 and its derivatives on [<sup>3</sup>H]methylphenidate binding

Compound	IC <sub>50</sub> (nM)	n <sub>H</sub>
GBR-12783	12.1 ± 1.3	2.7
2HD7.3	39.3 ± 4.7	1.7
2HD16.3	493 ± 94	3.0
2HD15.5	1677 ± 412	1.8

IC<sub>50</sub> = concentration required to inhibit 8-10 nM [<sup>3</sup>H]methylphenidate binding by 50% when incubated for 30 min at 0°C. IC<sub>50</sub> values are means ± SEM derived graphically from three separate inhibition curves generated using 5-7 different concentrations. n<sub>H</sub> = Hill coefficient; values shown are the average of two separate experiments conducted as described above.