SYNTHESIS OF SOME PIPERIDINE DERIVATIVES OF POTENTIAL BIOLOGICAL INTEREST

Thesis submitted

for

the fulfilment of the

Degree of

Doctor of Philosophy

by

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"WHO SO WALKS IN THE PATH SEEKING KNOWLEDGE THERE IN, GOD WILL THEREBY MAKE EASY TO HIM THE PATH OF PARADISE

Abu-Hurayrah (MUSLIM)

Dedicated to my brother whose prayers and efforts elevated me to this stage

AKNOWLEDGEMENT

SUMMARY

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I shall be failing in my duty if I don't express my thanks to Prof. Dr. Darakhshan J. Haleem, Professor of Biochemistry, who has been most helpful in providing the facilities and guidance in carrying out the neurochemical studies of the compounds.

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Hanifa Shahnaz Siddiqui

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Present work includes the synthesis of piperidine derivatives having potential therapeutic activities.

Pethidine and morphine like compounds are well known for exhibiting agonistic as well as antagonistic activities. Molecular modifications of these compounds led to new opiates or narcotics analgesic. Piperidine is one of the most important heterocycles which exhibits many therapeutic activities due to its conformational flexibility. Minor alterations in the chemical structure have shown considerable change in the activities.

During the course of present work substituted phenacyl derivatives of methyl 4-hydroxy piperidine were synthesized. The analgesic and neurochemical activities were studied. The analgesic activities were determined by thermal method. Most of the compounds exhibited analgesic activity when evaluated by thermal method.

The effects of these newly synthesized derivatives are studied on brain mono amines levels. These compounds showed significant change in catecholamines and indolemines level in whole brain male albino mice at doses of 100 mg/kg body weight. The cytotxicity of these compounds was determined against Artimia salina by brine shrimp bioassay. Few derivatives exhibited promising cytotoxicity. The effects of these derivatives on smooth muscles (vivo and vitro) were also studied in Guinea pigileum and Rabbit Jejunum. Non of the compound showed activity upto a very bigh range 3mM.

Spectroscopic techniques such as 1H-NMR,EIMS, UV and IR were utilized for characterization and structure confirmation of the compounds.

INTRODUCTION

Many, if not more, ailments of the body cause pain, and the ability to diagnose the pain depends upon the knowledge of different qualities of pain. Pain is subjective sensation. It cannot be qualitatively measured with an accuracy, and there is no evidence that feels the same in others as it does in ourselves. However, it can be referred as a protective mechanism for the body, it occurs whenever any tissues are being damaged and cause the individual to react reflexly to remove the pain. Pain, then, can be defined as the "effect produced in consciousness by the arrival of nerve impulse generated by noxious stimuli, in the brain International Association For the Study of pain endorsed the definition of pain which describes the pain as an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage".

Treatment of pain with Drugs:

The drugs which bring about the insensibility to pain are called analgesics. Pain diminishes as the selective depression in the nociceptive mechanism takes place by analgesics. Analgesics, vary greatly, in their potencies and mode of action.

Traditionally analgesics were classified as weak and strong analgesic based on difference in the development of tolerance, dependence and

analgesic potency (**1**), this classification is less valid because a strong analgesic effect can be achieved with weak analgesics (**2**).

Lim and his coworkers have proposed another way of grouping analgesics based on the fact that the bradykinin cause pain in dogs (**3**). He classified the analgesics into three groups:-

1. Peripherally acting non-narcotics

2. Centrally acting non-narcotics

3. Centrally acting narcotics

This classification is further modified by Scheffer *et al.* (**4**). He classified the analgesics as opioid and non-opioid (narcotics and non narcotics). At present this classification is recognized in international nomenclature, therefore we will discuss analgesic drugs on the basis of this classification.

OPIOID (Centrally acting on Narcotic Analgesics)

The term opioid is used to specify drugs derived from opium (e.g. morphine,codeine etc.) and many semi-synthetic congeners of morphine like action. This term has also been used to refer to antagonists of morphine like compounds, as well as to receptors that combine with such compounds.

Opioid are further subclassified as under:

A. PARTIALLY SYNTHETIC ANALOGUES OF MORPHINE
B. TOTALLY SYNTHETIC MORPHINE LIKE ANALGESICS
C. OPIOIDS WITH MIXED ACTION (Agonist-Antagonist Partial agonists)
D. OPIOID ANTAGONISTS

PARTIALLY SYNTHETIC ANALOGUE OF MORPHINE

Various derivatives of morphine that had been prepared were basically the result of very slight modification on the molecule, such as esterification of

phenolic or alcholic hydroxyl group and as a result of similar minor changes, some very useful compounds were discovered with greater activity than morphine but also greater toxicities and adddiction tendencies. Some important members of this class are listed below:

- Codeine
- Ethyl morphine
- Diacetyl morphine (Heroin)
- Hydrocodone
- Oxycodone etc.

TOTALLY SYSTEHTIC MROPHINE LIKE ANALGESICS

Synthetic morphine like analgesics are further subclassified as follows:

(a) Phenylpiperidine and Piperidine Derivatives

Examples of this class include:

Pethidine (meperidine), Fentanyl, Sulfentanil, Carfentanil, Lofentanil etc.

Methadone (Amidone) Derivatives

The representative members of this class include:

Methadone, Isomethadone etc.

Morphinan Derivatives

Examples of this class:

Levorphanol, Recemorphan, N-methyl morphinan etc.

Benzomorphan Derivatives

The important members of this class are listed below:

Phenazocine, Pentazocine etc.

OPIOIDS WITH MIXED ACTIONS (AGONIST-ANTAGONIST OR PARTIAL AGONIST)

The main compounds possessing agonist – antagonist and partial agonistic activity are listed below:

- Nalbuphine
- Butorphanol
- Buprenorphine
- Meptazinol
- Nalmefene

PURE OPIOID ANTAGONISTS

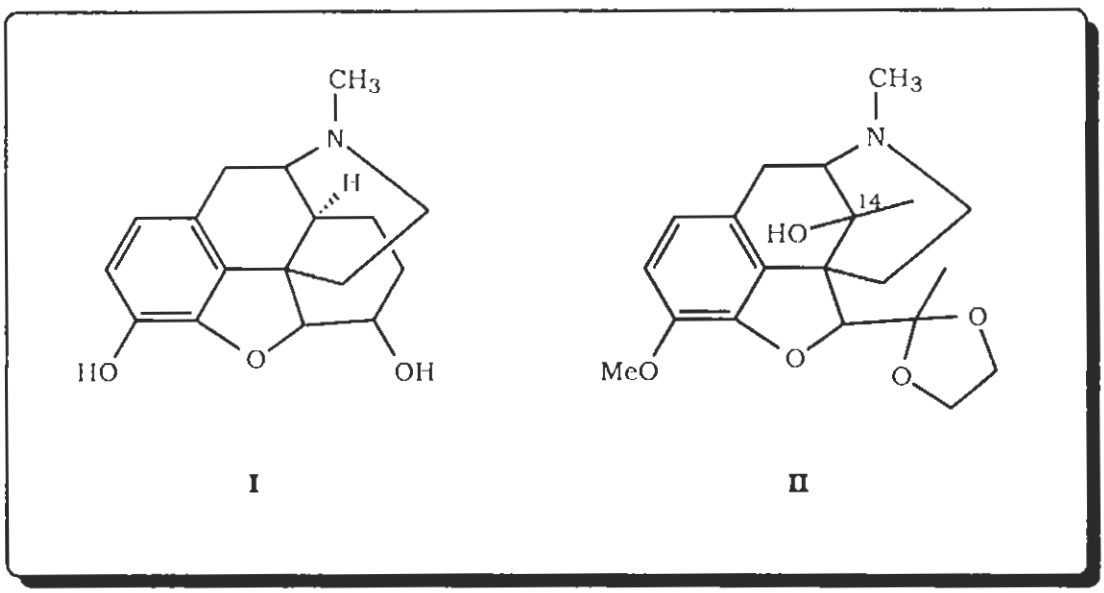
The compounds possessing antagonistic activity are:

- Nalaxone
- Naltrexone etc.

The fact that opium possesses pain-relieving properties has been known to man from very early times and has been used very effectively for the purpose. The active constituent responsible for this activity is morphine.

Although morphine possesses extremely effective analgesic property its use has to be limited in view of its certain undesirable effects of which "Physical Addiction" is most serious one. A considerable amount of research work has been directed towards they synthesis of its analogue completely devoid of this drawback or modified to a possible extent.

Surprisingly *trans* morphine I (H. Kugita, M. Takeda and H. Inoue) is found to be about 15 times less active as compared to morphine in the hot plate test, although its isomer morphinan and benzomorphan are shown to be better analgesic.



I II

Nordihydrodeoxymorphine II as compare to morphine has shown enhanced activity by the hot plate test when injected subcutaneously (J.E. Villarreal, and M.H. Seevers). The activity is completely lost in the hot plate test if the N-methyl group is replaced by NH_2 group (A. Modiri, J.G. Cannon and S.Y. Yeh).

Codeine, the nearest structurally similar compound is in still extensive investigation for its analgesic and antitussive effect (N.B. Eddy, H. Freibel, *et al.).* 14-hydroxy morphine and the same analogue of dihydromorphine are known to be moderately potent (U. Weiss, and S.J. Daum) while the 6-ethane ketal is more potent than morphine in mice and rat on oral administration (D.J. Barron, P.L. Hall *et al.).* The reason for this increase in the potency has been attributed to the hydrogen bonding in 14-hydroxy codeine III and its dihydroanalogue (T.B. Zalucky *et al.)* Esterifiction of 14-hydroxy group also results in the increase in potency (W.B. Beckett *et al.).* e.g.. IV. The increase in the size of R group of the ester increases the potency upto n-hexyl group after which it decreases benzoyl esters are not active, but phenacyl cinnamoyl and related esters are highly potent. Fat solubility of these compounds Transport factor and conformational factors may play important role in this structure activity relationship.

Phenyl function of morphine is completely masked by its conversion to methoxy methyl group (A. Crum Brown *et al.)* resulting in the increase of ED_{50} value in rats to 28.8 mg/kg.

The morphine and its methochloride (its quaternary salt) lose its analgesic property when given systematically. The reason perhaps being that the completely ionised salt is not able to penetrate the CNS.

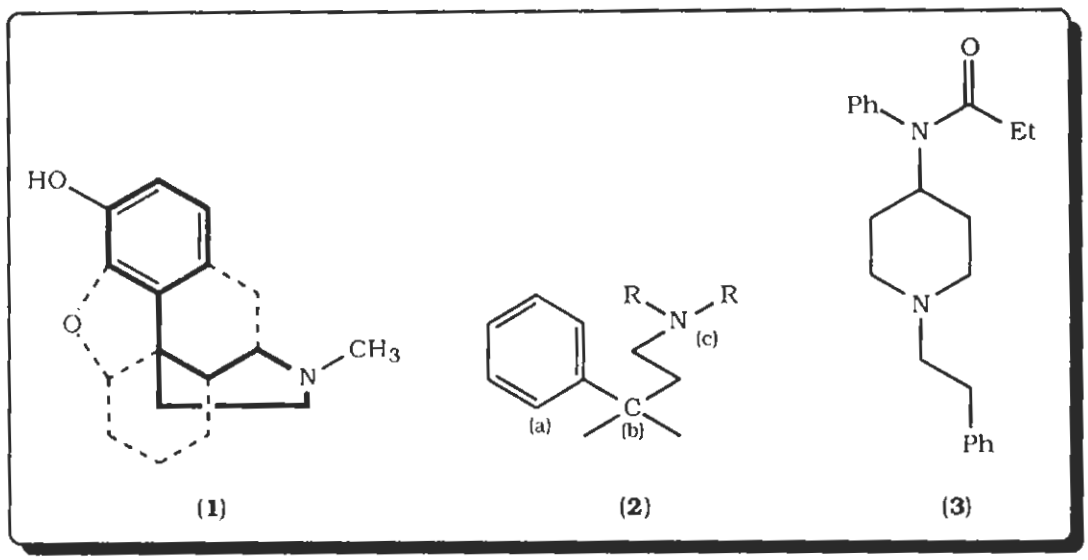
However when these drugs are injected intercerebrally they show analgesic activity. It is because of this reason that the morphine N-oxide after subcutaneous and interpericardial administration (M.R. Feunessy *et al.)* shows decrease in potency. Studies on new born rats (E.G. Way) indicate that the de-acetylated metabolites of heroin, morphine and 6-monoacetyl morphine (MAM) facilitate the availability of morphine at the CNS receptor. Codeine and morphine show reciprocal synergistic action in rat (T. Johannesson and L.A. Woods).

The observation (Jensen *et al.* 1943) that replacement of the ethoxycarbonyl group of pethidine by a propionoxy group, increases the potency, has since been confirmed in numerous cases. Such a change usually produces a 20-fold increase in activity, regardless of the nature of the *N*-substituent (Janssen and Eddy, 1960a). Most propionoxy esters are more active than acetoxy esters although Beckett *et al.* (1959) found the reverse to be true with esters of 1-phenethyl-4-aryl-4-piperidinols.

The results in case of replacement of *N*-methyl by *N*-aralkyl groups in esters of 4- aryl-4-piperidinols are qualitatively similar to those obtained in pethidine series. A large number of esters of 4-aryl-1-phenethyl-4-piperidinol have been prepared and each compound was found to be more potent than the corresponding N-methyl analogue (Beckett *et al.* 1959). Some 4-aryl and 4-aralkylpiperidinols, in contrast

to their esters, are CNS depressants of the tranquilizing type (e.g. haloperidol, **19**, Janssen *et al.* 1959d) rather than analgesics, 4-Acetoxy-1-isopropyl-4-phenylpiperidine is reported to have significant analgesic activity in mice but N-dimethylamino analogue (a hydrazine derivative) is much weaker despite the fact that the two *N*-substituents are of similar size (Beckett and Greenhill, 1961). Incorporation of the adamantyl moiety into the ester function of pethidine, giving (**20**) is claimed to be advantageous both in terms of potency and duration of action (Voldeng *et al.* 1968).

It was observed that activity requires an aromatic ring (1) attached to a quaternary centre (b) and a tertiary nitrogen (c) at a distance of two carbon atoms from (a). This rule has its exceptions too as fentanyl (3), for example, in case of such generalisation, does not come under this rule.



(1) (2) (3)

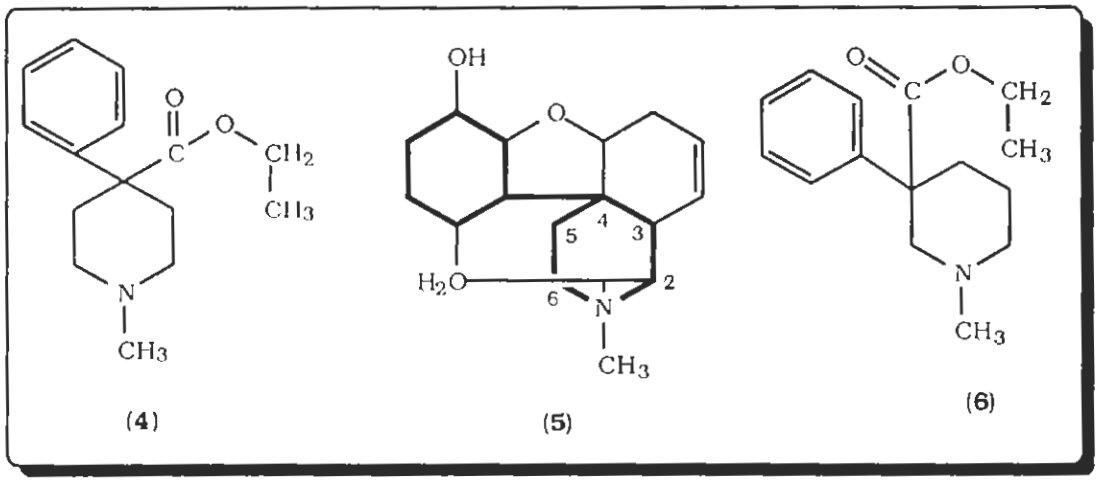
Brief study of synthetic fragments, indicates that morphine has been dissected in many possible ways to synthesize efficacious compounds inspite of detailed analysis of morphine molecules and its characterisation. Search for new analgesics preferable to morphine did not prove fruitful until Eisleb and Schaumann (1939) synthesized a large number of piperidine derivatives for testing them for spasmolytic activity; atropine was regarded as the parent structure. A number of atropine derivatives so synthesized, luckily showed marked * analgesic properties in addition to atropine like action.

A detailed study of molecular structure (**5**) then pointed out the fact that they constituted a fragment of the morphine molecule, namely 4-phenylpiperidine moiety, C-4 being quaternary. The two syntheses used

by Eisleb in the preparation of this series permitted a wide variation of structure without changing the synthetic approach. Approximately 40 piperidine derivatives containing in the 4-position, an aromatic ring and a number of unsaturated functions (keto, nitrile and ester) were tested, and those with the most favourable therapeutic index were chosen for clinical trials.

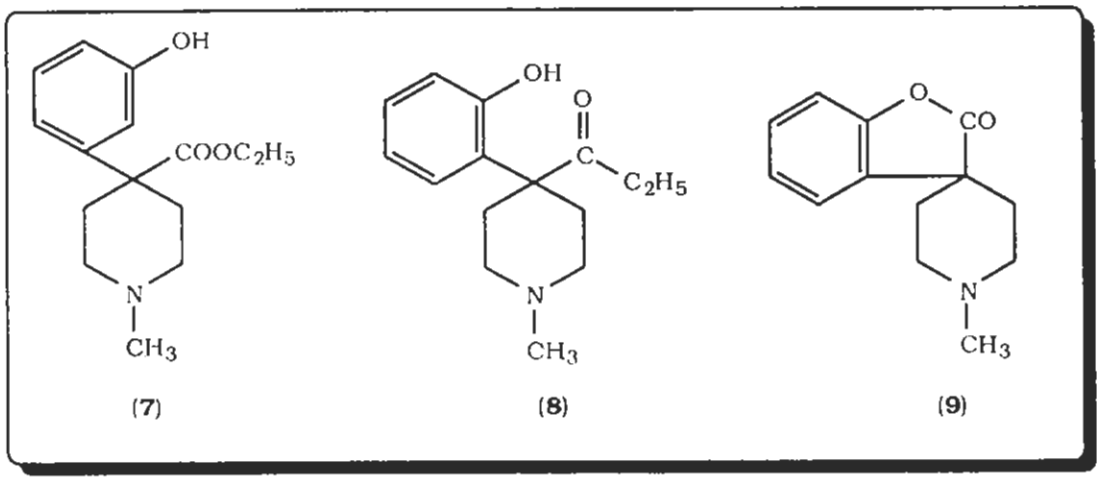
The most important substance to emerge was 1-methyl-4-carbethoxy-4-phenylpiperidine (Meperidine, **4**), with about 1/8 the potency of morphine in man (Lasagna and Beecher, 1954).

Beta pethidine (**6**) (Bergel *et al.* 1944), a structural isomer of meperidine with the phenyl and carbethoxy groups in the 3-position has been studied in the laboratory animals (Macdonald *et al.* 1956) and man (Glazebrook and Branwood, 1945). It has a low toxicity but is a less potent, shorter acting analgesic than meperidine.



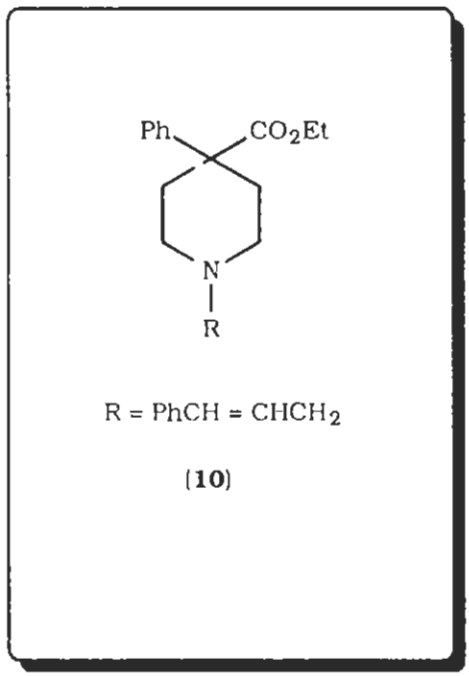
(4) (5) (6)

Earlier work on the structural modification of pethidine has been reviewed (Braenden *et al.* 1955) and advances upto 1957 have been surveyed by Beckett and Casy (1957). The most active analgesic of the meperidine series, ketobemidone (**8**) (Avison and Morrison 1950, Kagi and Miescher, 1949) resulted from a double modification of the molecule. The first consists in the introduction of a hydroxyl group into the *meta* position of the benzene nucleus and produces bemidone (**7**) (Macdonald *et al.* 1956) with activity comparable to the parent meperidine. In the second change carbethoxy group of bemidone is replaced by propionyl to give ketobemidone, which is 20 times more potent than meperidine. Another interesting modification of meperidine has been presented in 4-(6-hydroxy-3-methylphenyl)-1-isonipecotic acid lactone (**9**). This internal ester showed considerable activity in man (Spielman, 1950).



The structural variation includes replacement of *N*-methyl by other groups, notably phenyl alkyl, brought about in most cases by alkylation of norpethidine with the appropriate alkyl halide. These studies probably stem from the observation made by Perrine and Eddy (1956) that N-phenethyl norpethidine is twice as active as pethidine in mice. Elpern, Gardner and Grumbach (1957) examined the effect on activity in rats of lengthening the alkyl chain between the ring nitrogen atom and the aryl group of chain branching and of the influence of substituents in the phenyl ring.

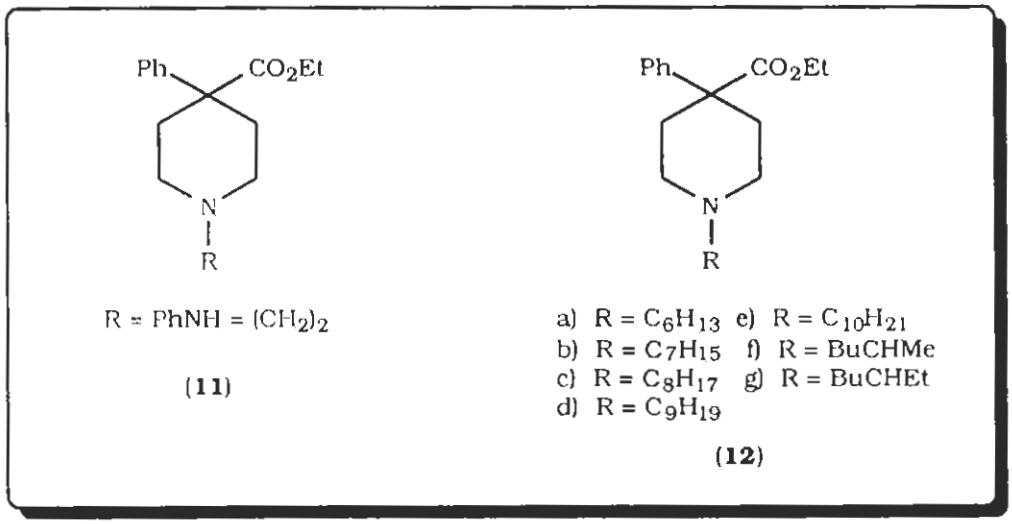
In the study of *N*-substituents mentioned above (Elpern *et al.* 1957), activity was further increased when the three carbon chain contained a double bond (*N*-cinnamyl analogue, **10**) but this is lost when a triple bond is included.



R = PhCH = $CHCH_2$

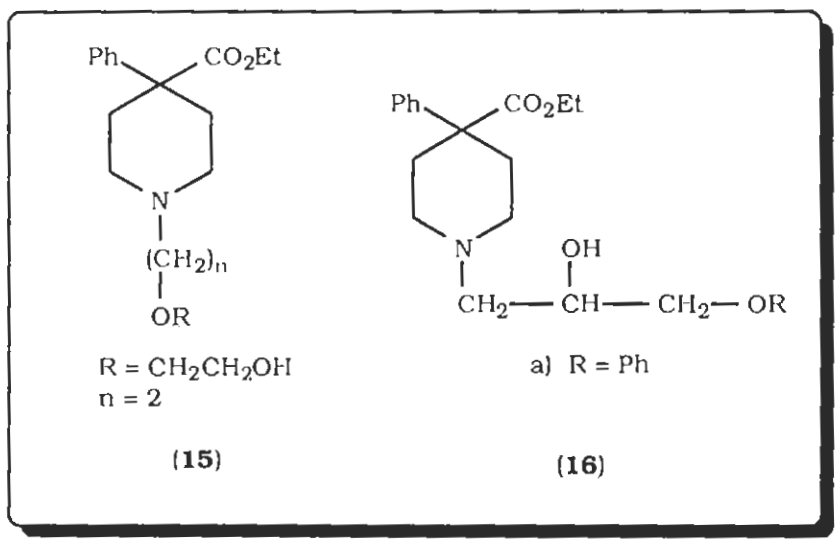
(**10**)

A further series of compounds (**11**) was prepared (Elpern *et al.* 1959) in which an imino group was placed between the aryl and alkyl portion of the aralkyl substituents; majority of these compounds showed very high activities. Another paper (Elpern *et al.* 1969) relates tonorpethidine substituted with long chain alkyl group (**12**); these compounds were highly active in mice, and activity did not depress by alpha branching.

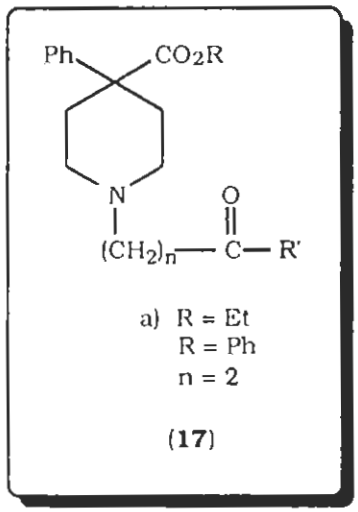


An examination of *N*-substituted norpethidines bearing alkyl groups having terminal oxygen functions (Frearson *et al.* 1958 and 1960) derives from the fact that while 2-morpholinoethylnorpethidine (**13**) and its sulphur analogues possess marked analgesic potency, replacement of oxygen (or sulphur) in the heterocyclic residue by carbon or nitrogen gives inactive compounds. This indicates that the presence of an oxygen or sulphur atom at some distance from the basic centre is desirable in this series of pethidine analogues (Anderson *et al.* Millar and Stephenson, 1956). The compounds prepared have the general formula (**14**). In the alkyl series, the 2-ethoxyethyl and 4- ethoxybutyl compounds are the most potent, being 5 and 10 times as active as pethidine respectively (Blair and Stephenson, 1960). The phenyl and benzyl analogues (**14a,b**) are about 7 times as active as pethidine.

norpethidine (Boggiano *et al.* 1959, **16**). This series is unique in having 2-hydroxy group which is shown to be important for analgesic action since the propanediol derivative (**16a**) is reported to be twice as active as the 3-phenoxypropyl analogue (Blanchi and David, 1960).



Study of aralkylnorpethidine having alkyl and aryl chains separated by a carbonyl group (Janssen *et al.* 1959a and 1960b) giving rise to Mannich bases (**17**) revealed that highest activity was found in 2-propiophenone compound (R 951, **17a**), which is 60 and 200 times more active than pethidine in mice and rats respectively (Janssen *et al.* 1959a). Increase of the alkyl chain length to three carbon atoms is supposed to reduce potency and substitution in the aryl group (R') has also similar effect although the decrease is small with the m-fluoro derivative.

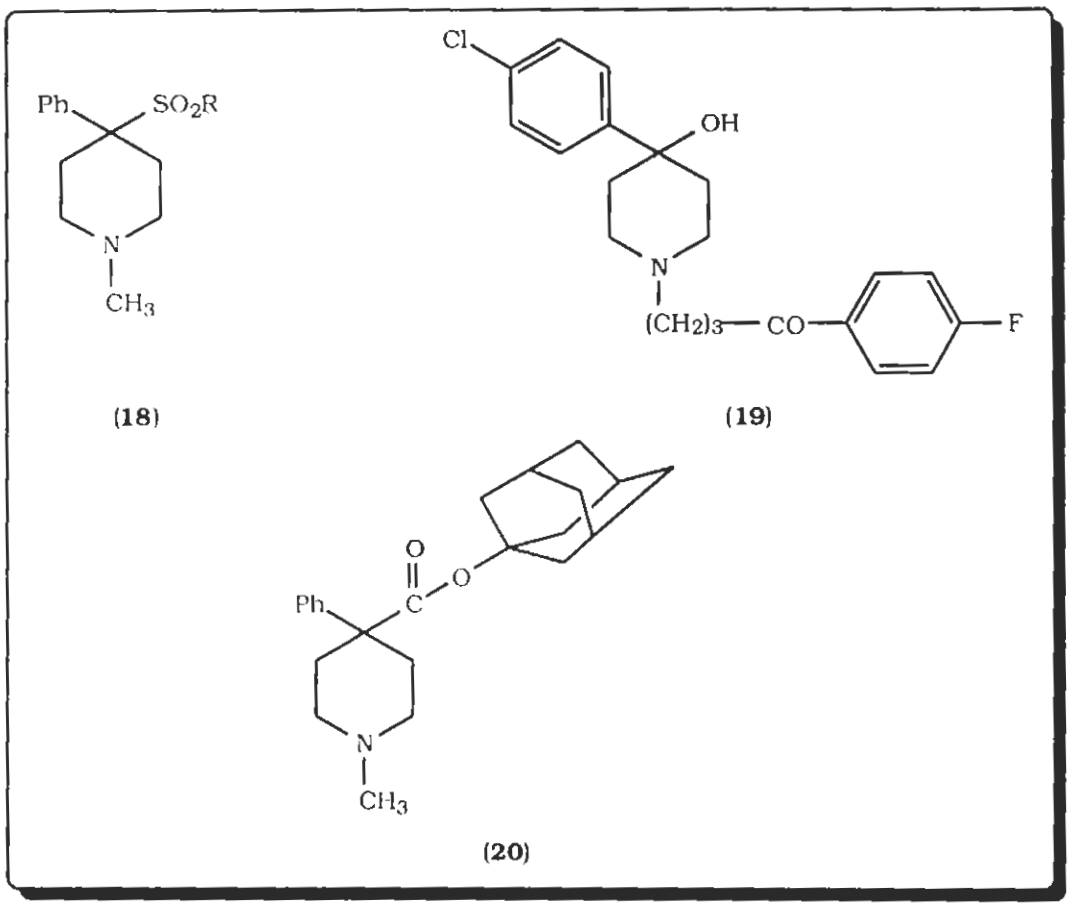


a) R = Et
R = Ph
n = 2

(17)

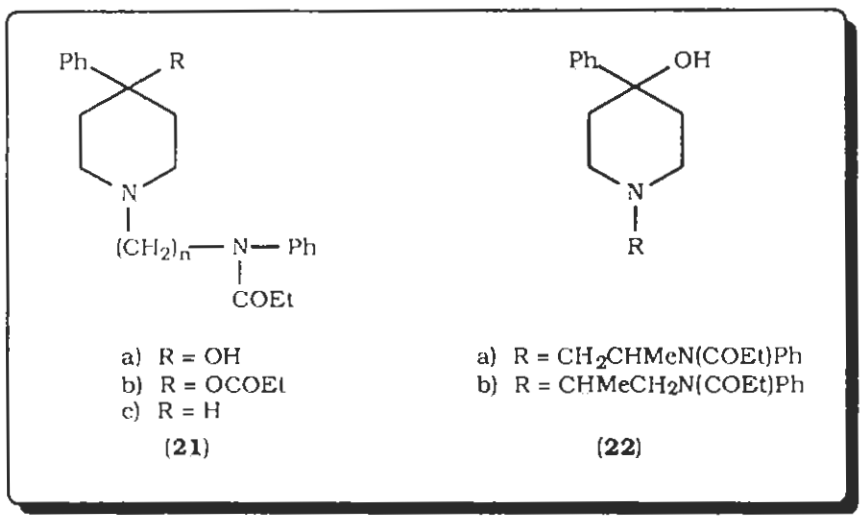
By varying the ester group (R) activity increases from methyl to ethyl, and then decreases rapidly with increase in chain length to butyl (Janssen *et al.* 1959c). The secondary alcohol derived reduction of the ketone to secondary alcohol increases activity than its precursor, but its acetyl derivative is less active than the ketone. In the butyrophenone series, the ketone and derived alcohol have similar activities (Janssen and Eddy, 1960a).

A group of Swiss workers (Buchi *et al.* 1952 and 1953) studied the series of 4-alkylsulphone pethidine analogues (**18**) and found a number of these to be as effective as pethidine in mice in test for analgesia. Some non-basic derivatives were also reported to have activities that of pethidine.



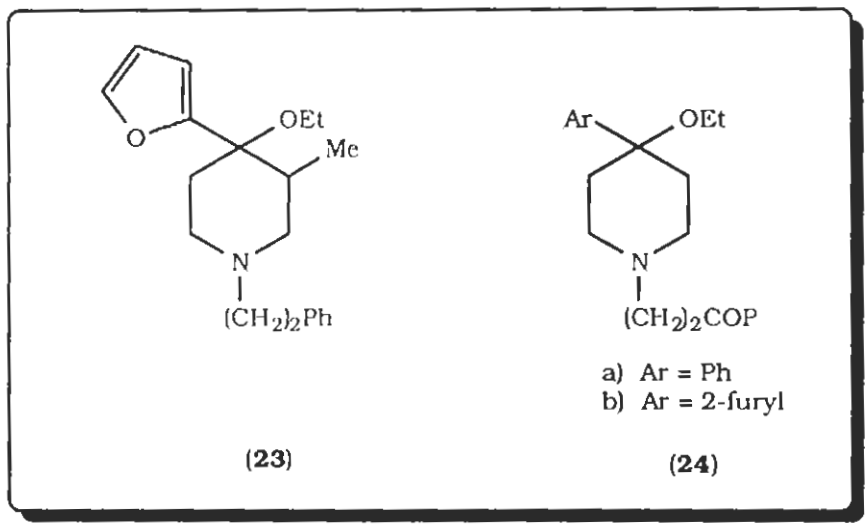
Normally *t*-alcohols corresponding to reversed ester analgesics are inactive (Beckett and Casy, 1965). The free alcohol (**21a**) however, isolated during attempts to prepare the *O*-propionyl ester, is highly potent in rats and its activity is in fact reduced on esterification (**21b**). (Carabateas *et al.* 1963), *N*-acetyl, *N*-butanoyl and 3-methyl analogues of (**21a**) and the 4- phenylpiperidine (**21c**, 4-OH removed) are all

significantly active. Even higher potency levels are reached in branched chain congeners (**22a-b**) the activity of former being noteworthy (Francher *et al.* 1964). Analogues of 22 with OH replaced by hydrogen are less active, but still more potent than morphine.



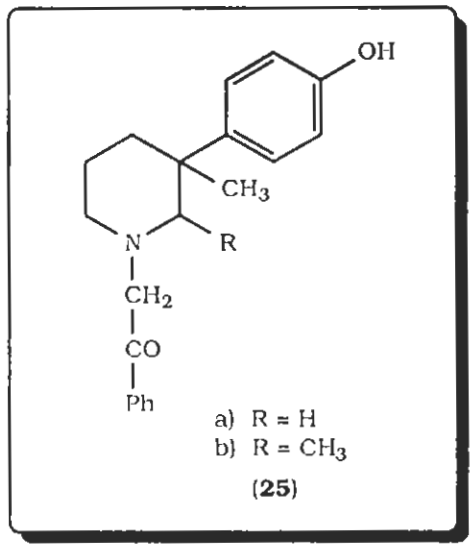
The morphine-like potency (Casy *et al.* 1961) of the 4-(2-furyl) ether (**23**) raises the question of the general acceptability or otherwise of a 4-alkoxy group as the C-4 oxygen function in the 4-phenylpiperidine analgesics. Comparison of the relative activities of several 4-phenyl and 4-(2-furyl) pairs such as **24** shows that the heteroaryl group is not essential feature of active 4-alkoxy-4-(2-furyl) piperidines. Hence, 4-alkoxy groups fulfill structural requirements for activity in 4-

phenylpiperidines although not so effectively as 4-acyloxy functions (Casy and Armstrong, 1965).



(23) **(24)**

Some 3-phenylpiperidine derivatives with significant analgesic activities have been reported (Kugita *et al.* 1963 and 1965). The most active members, for example, **25** have *N*-phenacyl or *N*-phenethyl substituents and their action is antagonized by *N*-allyl congeners; the latter also antagonize morphine, a result in contrast with the properties of *N*-allyl analogues of pethidine and its reversed esters (Casy *et al.* 1968). The derivatives of 3-carbethoxy-3- phenylpyrrolidine and piperidine lack both agonist and antagonist activities (Jacoby *et al.* 1974).

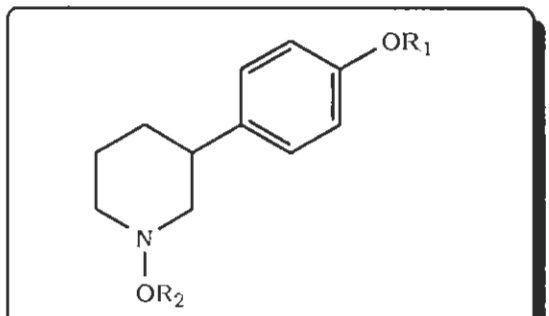


(25)

Much interest has been focused on the physiology and pharmacology of the dopamine (DA) auto receptor agonists during the last two decades, for example, apomorphine, have shown to act preferentially on the auto receptors thereby reducing nerve impulse flow, transmitter synthesis rate, and release in the CNS (Skirboll *et al.* 1979). Functionally, stimulation of DA auto receptors results in the decrease of locomotor activity and exploratory behaviour (Strombom, 1975). x It has been suggested that compounds with selective DA-auto receptor stimulating activity may be of therapeutic value (Carlsson, 1977 and Roth, 1979).

Thirty compounds related to the selective dopamine auto receptor agonists 3- (3-hydroxyphenyl) *N*-n-propylpiperidine (3-ppp, **26**) have

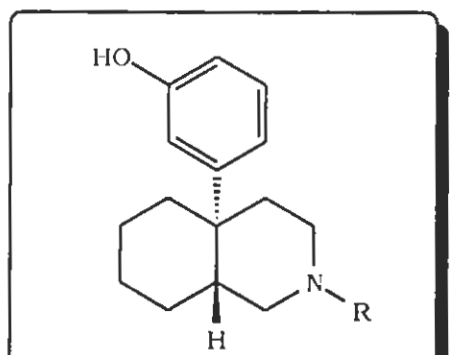
been synthesized and tests for central dopamine-auto receptor stimulating activity (Hacksell *et al.* 1981). The 3-(3-hydroxyphenyl) piperidine moiety seems to be indispensable for high potency and selectivity. Introduction of an additional hydroxyl group into the 4-position of the aromatic ring gives a compound with dopaminergic activity but lacks in selectivity for auto receptors. Another paper (Wikstrom *et al.* 1984) described the synthesis and pharmacological evaluation of enantiometric pairs of 3-(3-hydroxyphenyl) *N*-n-propylpiperidine (**26**) in order to examine their ability to interact with central dopamine (DA) receptors, particularly DA auto-receptors. In the total series, **26a** seemed to be the most interesting compound both from theoretical and therapeutical point of view and has been selected for extended pharmacological studies as a potential antipsycholic drug.



a) R_1 = H, [(S)-(-)-3-PPP]
R_2 = n-C_3H_5
(3-PPP)

(26)

Theoretical studies on 2-methyl analogues of 3-(3-hydroxyphenyl) piperidine, show diminished m-receptor affinity and analgesic activity, but demonstrated stabilization of the equatorial phenyl conformer (Lawson *et al.* 1988). Several constrained 3-alkyl-3-(3-hydroxyphenyl) piperidines have proved to be pharmacologically interesting: a recemic *N*-phenethyl *trans*-4a-aryldecahydroisoquinone (**27**) has 3-10 times the potency of morphine and high μ-affinity, while the 4a R, 8a R-N-cyclopropylmethyl has a mixed agonist/antagonist profile similar to pentazocine, with both μ and κ receptor affinity (Zimmerman *et al.* 1988).

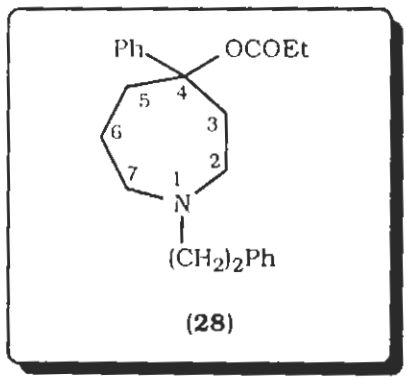


a) R = $(CH_2)_2Ph$
b) R = $CH_2OC_3H_5$

(**27**)

A study of ring contraction and expansion upon activity in reverse esters of pethidine together with other data show that analgesic properties

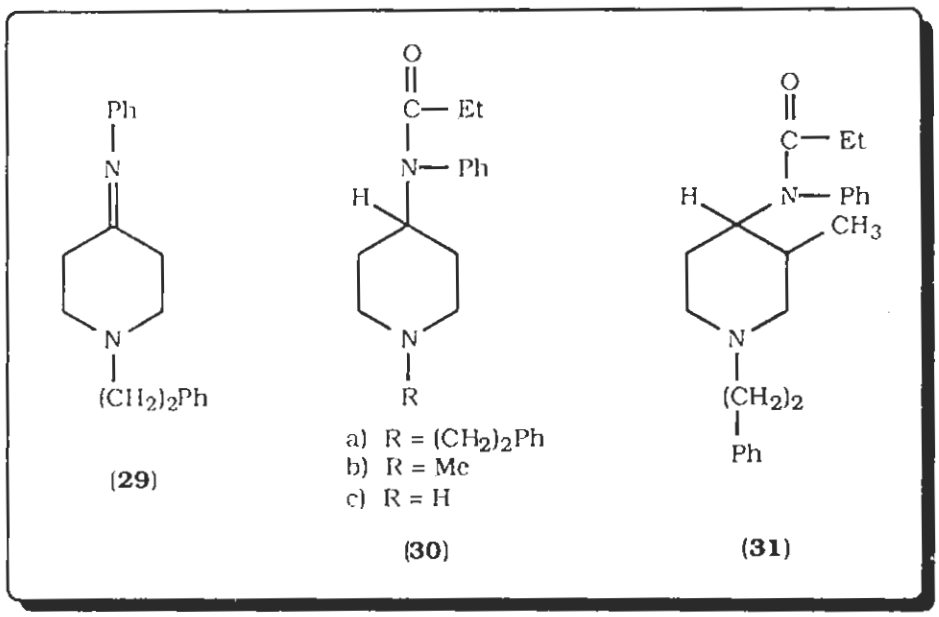
persists (although in reduced degree) in 7-membered ring of active piperidine derivatives but are disappeared or weak in 5-membered congeners (Casy *et al.* 1965 and Saify, 1971). The azacycloheptane (**28**), the most active non- piperidine derivative of the reversed ester series was a typical morphine-like analgesic, produced Straub tails and was antagonized by nalorphine, and tolerance developed towards its effects in mice.



(28)

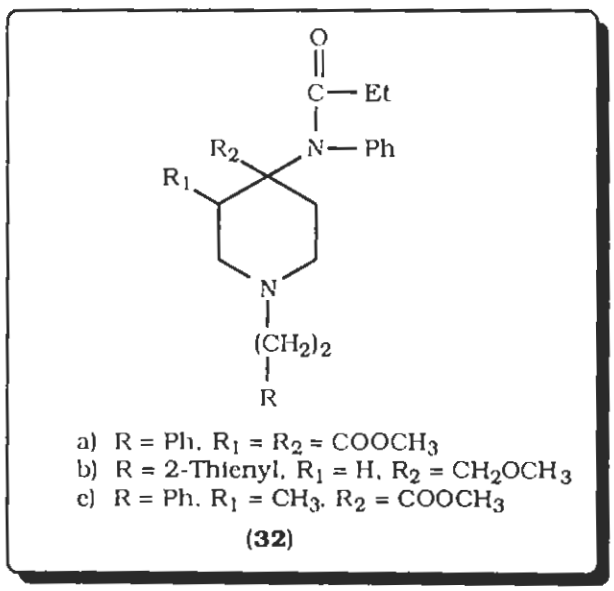
Reduction of Schiff base (**29**) formed between 1-phenethyl-4-piperidone and aniline, and acylation of the resultant dibase gives the analgesic having high potency (Janssen, 1964), fentanyl (**30a**). The *N*-methyl analogue of fentanyl (**30b**) has much lower potency than the parent (Casy, 1969). Riley *et al.* (1973) first reported 3-methyl fentanyl (31), prepared from a 4-anilinopyridine, which proved to be 10 times more effective than fentanyl itself in rats by the tail-flick assay. Cyclic

analogues of fentanyl in which the *N*-acyl group is joined to *N*-phenyl (Klein *et al.* 1975), and *N*-phenyl linked to C-3 of the piperidine ring (Berger *et al.* 1977), were both lack in analgesic properties.



Detailed investigation of the structure activity-relationship in the fentanyl meperidine series revealed that additional substitution of the amide nitrogen- bearing center resulted in still further enhancement of analgesic potency. Chemical modification of the fentanyl structure at the C-4 position of the piperidine ring proved to be successful approach (Van Daele *et al.* 1976). Thus introduction of a carbomethoxy group gave carfentanil (**32a**), whereas addition of a methoxy methylene group

coupled with isosteric replacement of the phenyl ring of the phenethyl substituent by a thienyl ring led to sufentanil (**32b**). Both carfentanil and sufentanil are very potent and long-acting analgesics. Stereospecific introduction of a methyl group at C-3 position of the piperidine moiety of the carfentanil molecule resulted in extremely potent and long acting compound lofentanil (**32c**).

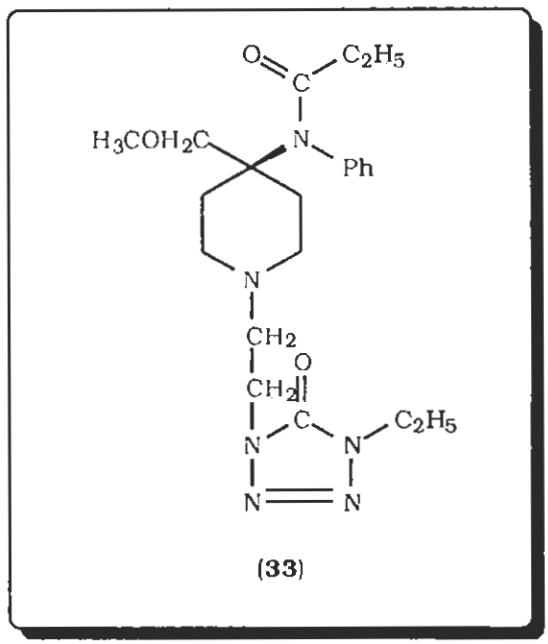


a) R = Ph, $R_1 = R_2 = COOCH_3$
b) R = 2-Thienyl, $R_1 = H$, $R_2 = CH_2OCH_3$
c) R = Ph, $R_1 = CH_3$, $R_2 = COOCH_3$

(32)

Janssen *et al.* (1986) synthesized a series of *N*-1,4-disubstituted, 1,4-dihydro-5 H-tetrazole-5-one derivatives of the fentanyl family. The ethyl derivatives (**33**), alfentanil (R-39209) was selected for extensive clinical investigations and as a result of its characteristic physicochemical and

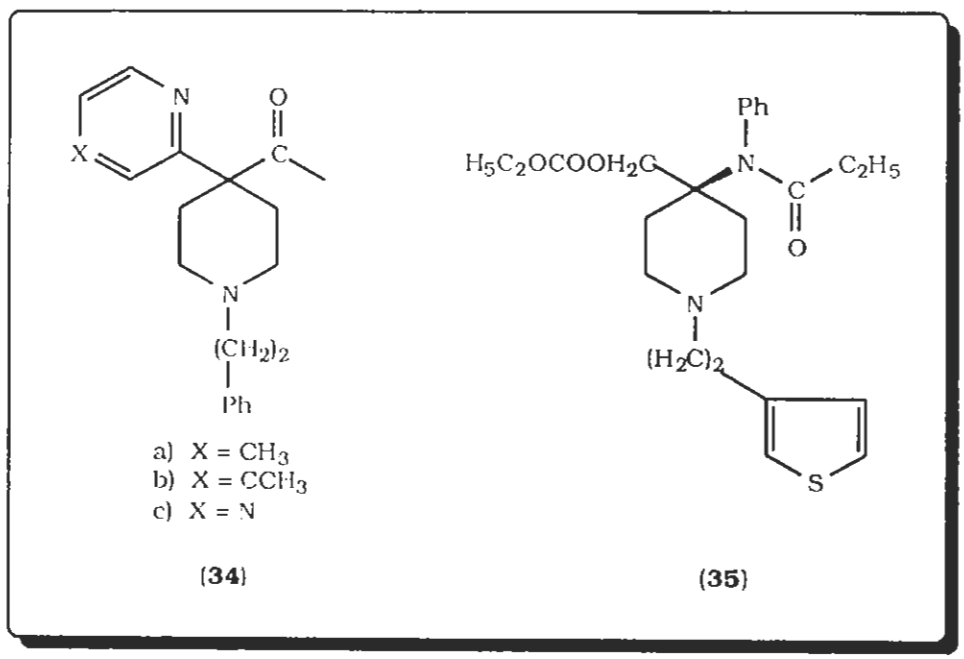
pharmacokinetic properties, placed this compound in a unique position within the class of clinically useful narcotic analgesics.



(33)

^{13}C-NMR studies on substituted 4-anilidopiperidines, which have a common calculated minimum energy conformation (Tollenaere and Janssen, 1988) have shown solution conformations similar to fentanyl (Brine *et al.* 1989). *Para* substitution on the aniline group of fentanyl gives derivatives with potencies greater than morphine, although less than fentanyl itself (Casy and Huskstep, 1988a). Heterocyclic substitution for the phenyl ring has led to a potent opiate agonist (**34a**) and a novel antagonist (**34b**), which reverses both morphine-induced

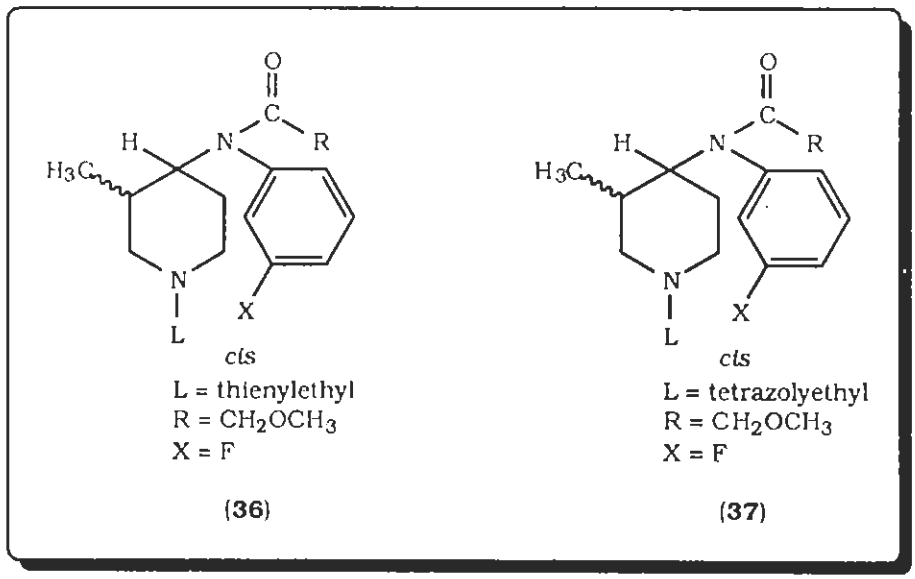
analgesia and respiratory depression (Bagley *et al.* 1989). Notably one 4-(hetero-anilido) piperidine (**34c**) possesses a good analgesic profile in several animals models, with little cardiovascular and respiratory depression compared to fentanyl. An anilinopiperidine 4-carbonate derivative (**35**) is potent and long acting (Colapret *et al.* 1989). Several 4-phenyl and 4-heteroaryl-4-anilidopiperidines possess high analgesic potency and favourable pharmacological profiles (Kudzma *et al.* 1989) and Feldman *et al.* 1991).



(34) (35)

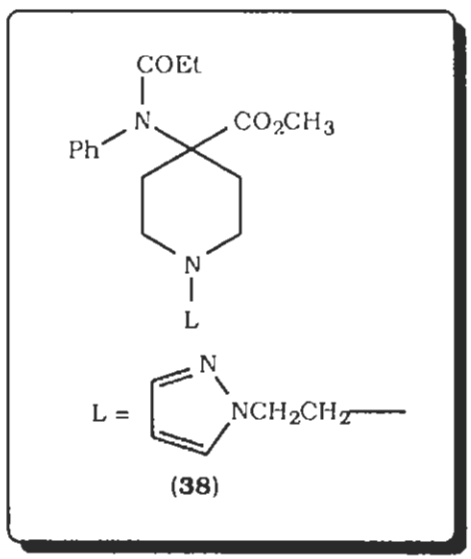
Lalinde *et al.* (1990) synthesized a series of racemic *cis* and *trans* stereoisomers of 3-methyl-4-anilidopiperidine in order to develop

therapeutically advantageous analgesics with rapid onset and short duration of action. The effect caused by changing propionyl group to a methoxyacetyl were studied, since this substitution is known to confer short duration of action in 4-anilidopiperidine analgesics (Huang *et al.* 1986). Among the resultant active compounds, *cis*-**36** was 13036 times more potent than morphine and 29 times more potent than fentanyl; however, the corresponding diastereoisomer *trans*-**36** was only 2278 and 6 times more potent respectively. Compound (Anaquest A-3331 HCl, Briefentanil, *cis*-**37**) was selected for further clinical evaluations.



Yet another similar attempt, a series of new 1-(heterocycloalkyl)-4-piperidinyl methyl esters and methyl ethers have been synthesized and

pharmacologically evaluated (Bagley *et al.* 1991). In the mouse hot plate test, the majority of compounds exhibited an analgesia (ED_{50} < 1 mg/kg) superior to that of morphine. Compound methyl 1-[2-(1H-pyrazol-1-yl)-ethyl]-4-[(1-oxopropyl)phenylamino]-4-piperidine carboxylate (**38**), which exhibited appreciable μ-opioid receptor affinity, was more potent and short- acting analgesic than alfentanil with less respiratory depression in the rat.

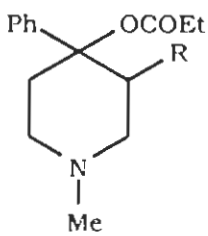


(38)

The effect of alkyl substitution in the piperidine ring of 4-phenylpiperidine analgesics has attracted much interest ever since the 3-methyl analogues of the reversed ester of meperidine were described in the late 1940s (Randall and Lehman, 1948). Since that time many 3-

alkyl and all possible *mono* and nongeminal di-c-methyl derivatives of reversed ester have been reported, and much data have accrued on potency variations among isomeric sets and their relative and absolute geometries. Consequences upon the results (Casy *et al.* 1982) a consistent stereochemical structure-activity pattern was developed on the basis of 4-phenylpiperidine ligands associated with the opiate receptors in the form of equatorial 4-phenyl chair conformations.

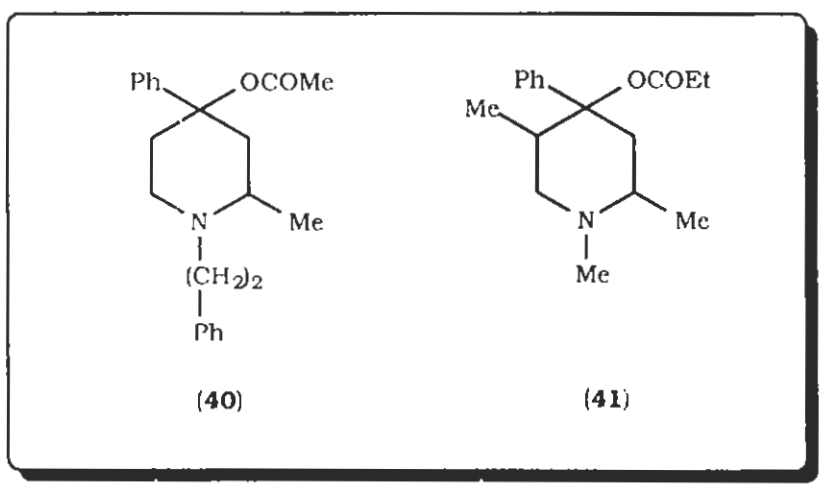
Methyl substitution in the 3-position is favourable and leads to the potent compounds, alpha and beta prodine (Randall and Lehman, 1948, **39**). The observed enhanced activity of alpha prodine over that of pethidine in animals has been confirmed in man (40-60 mg of alpha prodine is equivalent to 100 mg of pethidine, Bachrach *et al.* 1955).



a) Alpha pyridine, R = Me, 3-Me/4-Ph *trans*
b) Betal prodine, R = Me/4-Ph *cis*
c) Allyl prodine, R = $CH_2CH{:}CH_2$

(39)

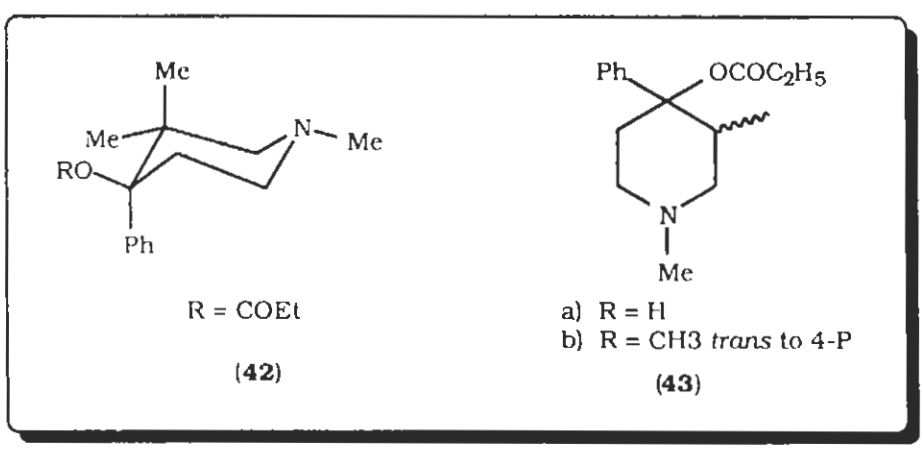
High activity is also associated with ethyl and N-propyl groups substituted in the 3-position but is lost with the large substituent (for example benzyl, McElvain and Barnett, 1956). The 3-allyl analogous (allyl prodine, **39c**) has been reported to be ten times as active as alpha prodine in rats but twice as toxic (Benson *et al.* 1957). The only example of 2-methyl substitution available (**40**) is 1/5 as active as the unsubstituted analogue; the latter (several times as potent as morphine in mice) is somewhat more active than the 2,6-dimethyl compound (Balon, 1959). 3,5-Dimethyl analogues are inactive while the 2,5-dimethyl compounds of Nazarov are highly active (Nazarov *et al.* 1949). Trimeperidine (promedol, **41**) is reported to be several times more active than pethidine in animals (Nazarov *et al.* 1952).



(40) **(41)**

Similarly, in another paper (Ahmed *et al.* 1985) described the preparation and resolution of 1,3,3-trimethyl-4-phenyl-4-(propionyloxy) piperidine (**42**) and the results of the antinociceptive activities of the products by hot plate (mice) and tail withdrawal test (rats) are shown to support the idea based on the stereochemical structure-activity relationships of C-methyl derivatives of the reversed ester of meperidine.

4-Phenylpiperidine analgesics which have piperidine chairs with an axial phenyl substituent as favoured conformation are rate but not unknown. Since these analgesics are complicated by conformational difference among the substituents, therefore structure-action relationships in substituted 4-phenylpiperidines are not easy to establish. The influence of stereochemical factors in reversed esters of 4-hydroxypiperidine is reflected in the wide difference in potency found among geometric

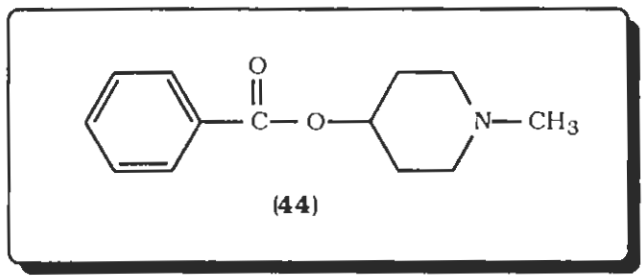


isomers such as trimeperidine and isopromedol (Mashkovskii and Abramova, 1956) and alpha and beta prodine type compounds (Beckett *et al.* 1959 and Randall and Lehman, 1948).

Much work on the stereochemistry of analogues alkylated at piperidine ring carbon atoms has been reported which helps in building a picture of the complex requirements of opiate receptors in relationship to 4-phenylpiperidine ligands. It has long been known that potency of parent ester (**43**) is slightly changed after insertion of 3-methyl trans to 4-phenyl (as in α-prodine, **39a**) but is elevated several folds when the substituent is cis to the aromatic group (as in β-prodine, **39b**). It has only recently been established, however, the case of methyl is unique and in pairs with larger alkyl substituents, the α-isomer (*trans* 3-R, 4-ph) is the more potent (Iorio *et al.* 1975). Receptor affinities measured by determining the concentration of 3- alkylated ester to displace 50% of specifically bound (H) dihydromorphine from rat brain homogenates have confirmed the higher affinity of α-over β-(*cis*-**43b**, R=Me) and a-over β (*trans*-**43b**), and results correlate well with analgesic potencies (Iorio and klee, 1977).

Substituted benzoic acid ester of 1-methyl-4-piperidinol (**44**) (Cheng *et al.* 1982) showed analgesic activity when assayed by mouse hot plate method, the more potent one falling in the morphine-codeine range, but

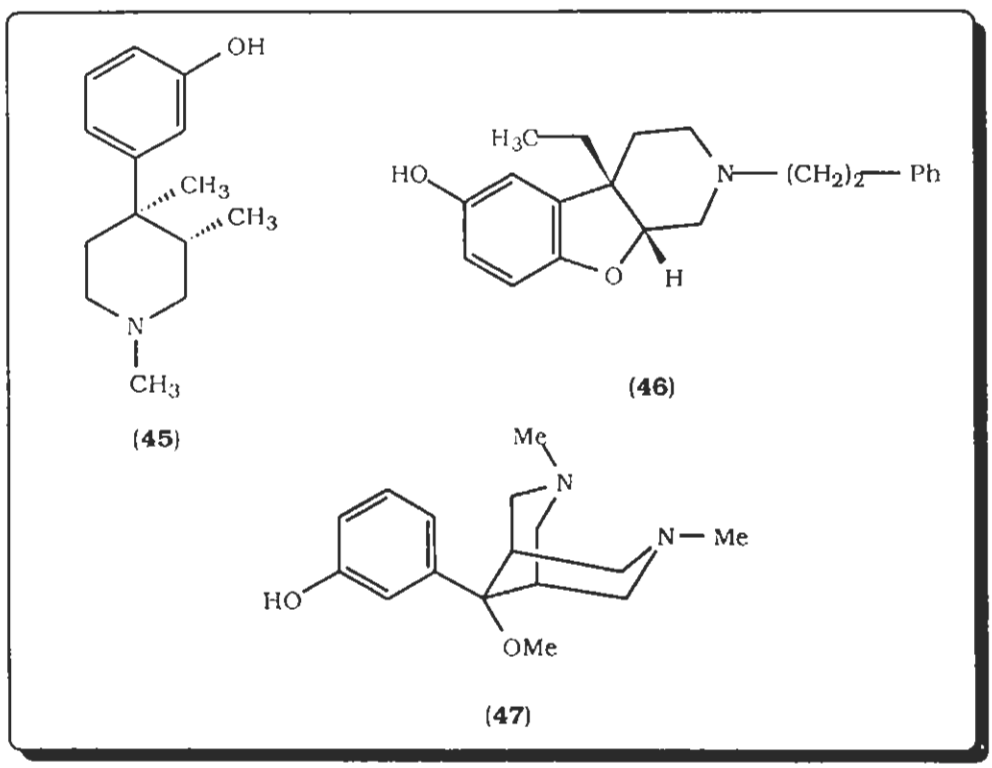
generally, they displayed no morphine like physical dependence liability in monkeys.



(44)

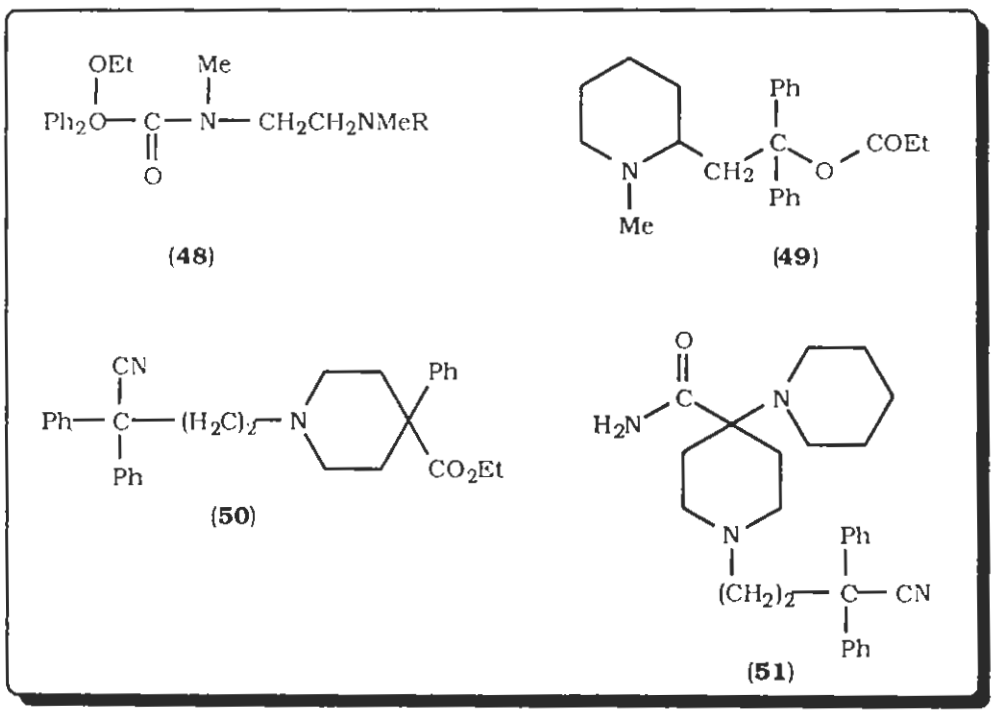
Research interests in piperidine analgesics have been focused on both experimental and theoretical studies in various alkyl, aryl and anilido structural classes. Energy conformational calculation and X-rays crystallography on a series of flexible 4-alkyl-4-(3-phenyl)piperidines have been used to describe a "Universal phenyl axial pharmacophore" which gives high affinity μ-receptor binding (Loew *et al.* 1988). NMR spectral studies have confirmed that 4-alkyl-4-(3-hydroxy or 3-methoxyphenyl) piperidines preferring the axial 4-aryl chair conformation are opiate agonists, whereas an antagonist was found to favour an equatorial 4-aryl chair (Casy *et al.* 1989), also preferred in C-4 hydrogen and C-4 reversed ester analogues. Picenadol (**45**), a mixed opiate agonist/antagonist without psychominetic effects, is being studied clinically for postoperative pain (Casy, 1988b). Among constrained 4-phenylpiperidines, the benzofuropyridine (**46**) has high

antinociceptive activity *in vivo* with a 2000- fold μ/κ-selectivity (Hutchinson *et al.* 1989) and 3-hydroxyphenyl substituted diazabicyclanes (**47**) are significantly more active than morphine as analgesics in mice (Salva *et al.* 1986). Novel N-butyrophenone prodine analogues have displayed opiate analgesic and neuroleptic activity (Iorio *et al.* 1987).



A number of novel structures containing quaternary diphenyl carbon centre, as in methadone, have been described (Casy, 1969). Certain

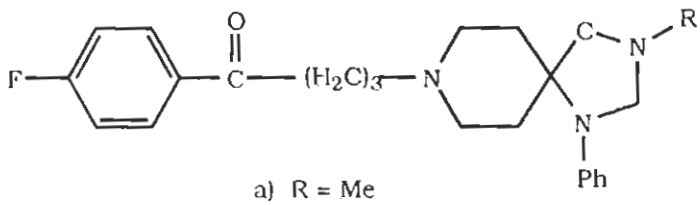
basic amides (**48**) of *O*-ethylbenzilic acid are active by mouth in mice and rats. The most active members are somewhat more potent than pethidine and carry β-arylethylamine *N*-substituents such as phenethyl and 2-(or 4) pyridylethyl, structurally the amide (**48**) bear some resemblance to diampromid and other basic anilides but are best considered as mild analgesics since their effective oral dose in man is high (150 mg, Krapcho and Turk, 1963). The derivatives (**49**) in which part of the methadone side chain has been incorporated into piperidine ring, is also reported as a potent analgesic (Newberne *et al.* 1967).



Janssen has linked the cyanide precursor of normethadone to norpethidine to produce diphenoxylate (**50**), this complex is not an analgesic but has the constipating action of morphine derivatives and is used as antidiarrhoeal agent (Janssen *et al.* 1962a,b and 1959b). The related 4-aminocarboxamide (**51**), piritramide (pirinitramide), obtained from *N*-benzyl-4-piperidone via the cyano-amide is an analgesic however, and is twice as active as morphine in mice (Janssen *et al.* 1961).

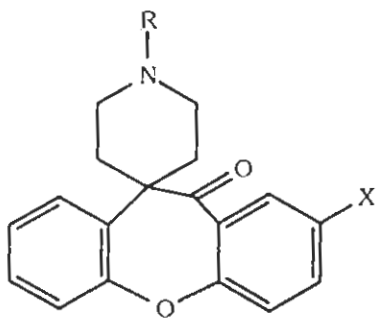
Several spiranes such as **52**, obtained by condensing 4-anilino analogues of **51** with formamide (Janssen, 1962c and 1965) have low hot plate ED_{50} values in mice; behave as powerful chlorpromazine-like sedatives in mice and monkeys. Although significant analgesic activity has been reported for a number of psychotropic agents with linear, tricyclic structure (Witiak *et al.* 1976, Davis *et al.* 1967 and Jilek *et al.* 1965), compounds of this type, in general, have rarely been investigated clinically as analgestics because of their diverse pharmacological actions. Ong and Profitt (1979) synthesized a series of 10,11-dihydro-11-oxospiro(dibenz(b,f)oxepin-10,4'-piperidinel derivatives (**53**) and evaluation was carried out for analgesic activity in the PQW assay and the tail flick test in mice. The compound **53a**, when administered orally, was equipotent to morphine in protecting against mouse writhing. Spiro

analogues (**54**) of the potent narcotic ketobemidone have been prepared and found to be devoid of opiate activity (Rogers *et al.* 1980).



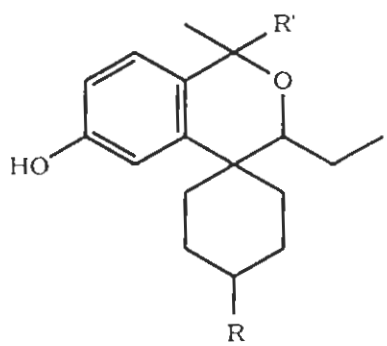
a) R = Me
b) R = COEt

(52)



X = H, F or Cl; R = H, alkyl cycloalkyl or aralkyl
a) X = F, R H

(53)

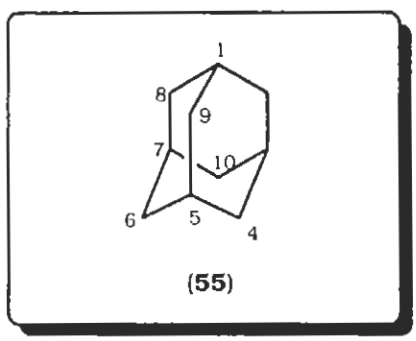


a) R = CH_3: R' = CH_3
b) R = $(CH_2)_4CH_3$: R' = CH_3
c) R = $(CH_2)_4CH_3$: R' = CH_2CH_3
d) R = $CH_2CH = CH_2$; R' = CH_3

(54)

The pronounced lipophilic nature associated with highly symmetrical, cage- like adamantane molecule (**55**, Landa *et al.* 1933) initiated a study of its influence on characteristics and biological potential of compounds

which contain this unique hydrocarbon moiety. It revealed (Rall and Zubrod, 1962) that the characteristics of a high degree of lipoid solubility, low extent of ionization and lack of plasma protein binding virtually ensure that a compound will enter brain and cerebrospinal fluid freely and attain equilibrium rapidly. Adamantyl group in the synthesis of diverse classes of medicinal agents (Gerzon *et al.* 1963 and 1967, Rapala *et al.* 1965), has been utilized in an effort that adamantane derivative might fulfill these criteria and thus penitrate the tissues and fluids in the CNS.

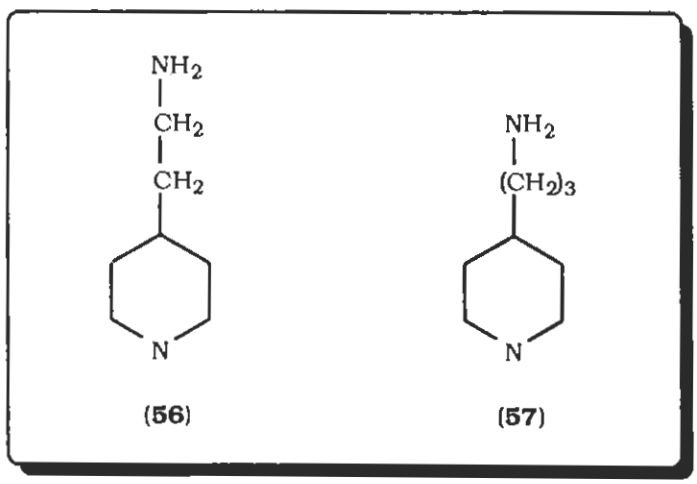


(55)

Several adamantane alkaneamines were prepared (Chakrabartil *et al.* 1974) and their activity in antagonizing reserpine-induced hyperthermia was compared with nortriptyline. The anti-Parkinson activity possessed by some of these potent amines in reversing the reserpin-induced catalepsy in rats was equivalent to or better than admantadine. Similarly, Henkel *et al.* (1982) synthesized a limited series of bridgehead

alkyl-dialkyl- and trialky-substituted amantadines and tested for potential anti-Parkinson activity as a dopamine (DA) agonists. The activity with in the series is dependent upon lipophilicity.

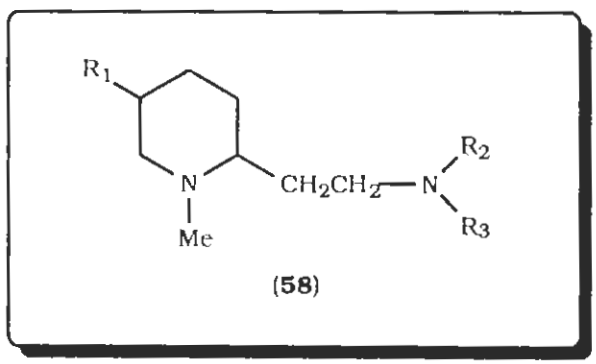
Apart from analgesics, a wide range of therapeutic agents are available in which piperidine nucleus is present. Specially hypotensive agents belonging to piperidine class of compounds are very important. The preparation of compounds of hexamethonium type in which the alkylene chain is incorporated in a cyclic structure was first reported by Norton (1953) and Phillips (1954). McMillan and Co-workers (1956) later prepared a variety of cyclic derivatives, both aromatic and heterocyclic, and found some compounds to possess hypotensive activity comparable to that of hexamethonium. Phillips (1957) has also reported on



(56) (57)

derivatives of 4-(2-aminoethyl) piperidine (**56**) and 4-(3-aminopropyl) piperidines (**57**). In each of these series certain of the diamines and bis-quaternary salts exhibited pronounced ganglionic blockade in cats.

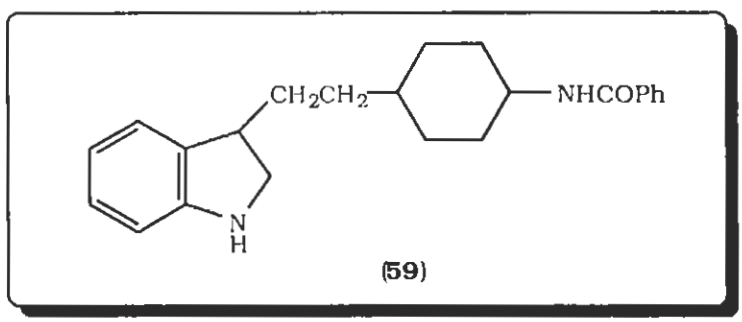
Shapiro and co-workers (1958 and 1960) have shown that the introduction of an oxygen atom into the alkyne side chain of structures of the type prepared by Phillips may also produce compounds having hypotensive notably dialkylaminoethoxy)methyl-1-methylpiperidines and 2-(3-dimethylaminopropoxy)-l-methylpiperidines, which show equal or better activity as free amines than as their his-quaternary salts. A series of l-methyl-2-(2- aminoethyl) pipericline (**58**) and their *bis*-quaternary ammonium salts have been prepared, evaluated and found hypotensive agents of the ganglionic blocking type (Minor et al, 1962).



(**58**)

The continuing interest in the indole derivatives incorporating a tryptamine residue as potential antihypertensive agents stemmed from

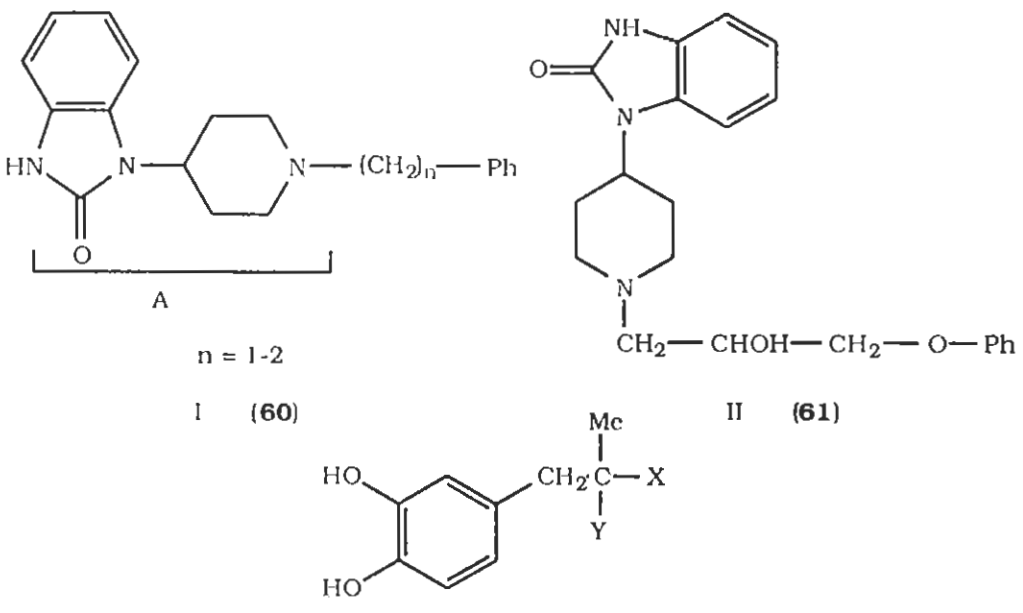
the work of Archibald *et al.* (1970) with 1,4-bis (indolylethyl) piperidine. In that series, the indolylethyl moiety attached to the piperidine 4-position was not an essential feature for retention of antihypertensive activity. It could, for instance, be replaced without any detriment by a .3-carbethoxy-2,4-dimethylpyrrol-5-ylethyl group (Archibald *et al.* 1966). While retaining the indolylethylpiperidine of the earlier series and varying the 4-substituents of the piperidine ring resulted in the discovery of 4-benzamidopiperidine derivative indormin, **59**) as a potent hypotensive agent (Archibald *et al.* 1971). This compound, then became the prototype for extensive synthetic programme designed to investigate structure-activity relationships and to optimize activity.



(59)

Many compounds having a structure which includes three or four methylene groups between a phenyl ring and the nitrogen atom of a piperidine ring have been synthesized in attempts to find new neuroleptics. However, only a few examples of Type I and II (**60** and **61**)

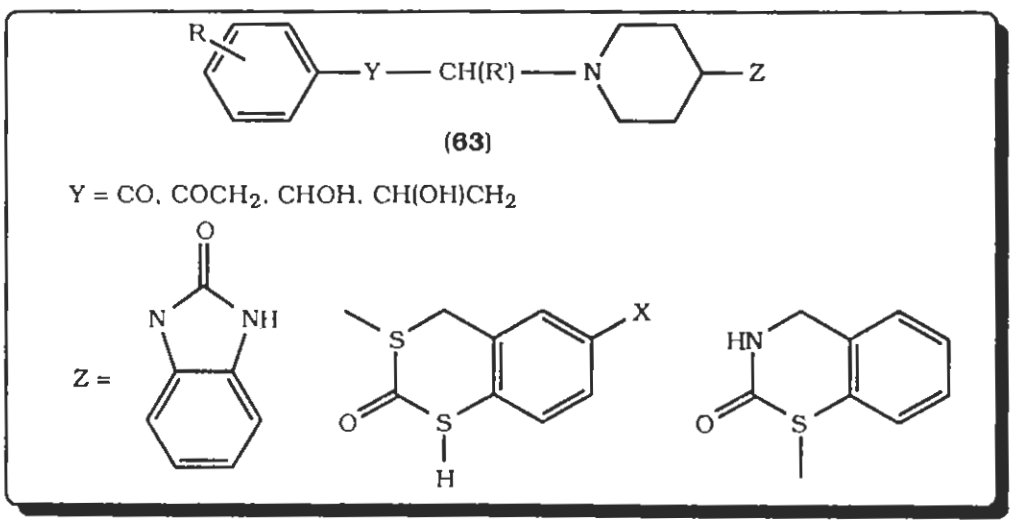
compounds are found in the literature (Janssen, 1967). Obase *et al.* (1982) designed compounds of the type III (**62**) on the basis of consideration that the bioavailability of methyldopa is known to be very low (Seriabine *et al.* 1980) and methyldopa derivatives (which are more efficiently absorbed) might show higher antihypertensive potency and secondly, methyldopa is widely thought to be a centrally acting antihypertensive agent, and the 4-pipericlylbenza-amidazolinone group



is likely to have high affinity for CNS. Benzamidazolinone derivatives of type III which contain a methyldopa moiety showed only moderate antihypertensive activity in three hypertensive rat models.

In a similar attempt, Takat *et al.* (1985a) prepared a series of piperidine derivatives (**63**) with various heterocyclic rings at the 4-position and tested for antihypertensive activity and other biological activities. The antihypertensive effects of the present compounds in the spontaneous hypertensive rats were less potent than those of previously reported compounds.

Preparation of a series of 1 and 3-(1-substituted 4-piperidinyl) 1,2,3,4-tetrahydro-2-oxoquinazolines for antihypertensive studies showed that



among the compounds tested, 1-(2-hydroxy-2-oxo-3-quinazolinyl) piperidine derivatives were generally the most effective in lowering blood pressure in SHR (Takai *et al.* 1985b).

Several reports have appeared on antihypertensive piperidine derivatives (**64**) with a heterocyclic ring in the spiro form (Maillard *et al.* 1970), 1972, 1973 and 1974, Klioze and Novick 1978, Caroon *et al.* 1981, Clark *et al.* 1983). Takai *et al.* (1985c) selected the spiropiperidine for further modification and resulting derivatives were evaluated for antihypertensive activity. Most of the compounds synthesized in this study showed strong hypotensive activities both in SHR and in normotensive rats. Moreover, among these compounds, several were found to produce a very large and long lasting decrease in blood pressure.

Very potent and competitive β-adrenergic antagonists (**65**), derived from (aryloxy) propanolamine β-blockers through introduction of a piperidine ring were introduced by Mauleon *et al.* (1988). This structural variation does not seem to markedly affect neither potency nor cardioselectivity, although one derivative was significantly more potent than propanalol.

During the course of present work the following objectives were kept in mind;

- Synthesis of the N-methylpiperidine derivatives by quaternization with substituted phenacyl halides.

- Collection and purification of the derivatives.

- Elucidation and confirmation of the structures of the newly synthesised derivatives by spectral studies which include UV, IR, EIMS and ^{1}H-NMR.

- Assessment of the biological potential of the synthesised derivatives. The analgesic activity of the derivatives was determined by mouse tail immersion method which is a thermal method of antinociceptive testing.

- Some of the derivatives were also evaluated for their effects on brain biogenic amines via neurochemical studies using whole brains of mice.

- All the derivatives were also screened for cytotoxicity by using brine shrimp bioassay technique.

- Some derivatives were also screened for their effects on the spontaneous activity of isolated rabbit jejunum and for their calcium channel blocking activity.

- The ultimate aim of the present study was the synthesis of simple quaternary derivative of N-methylpiperidine with optimum therapeutic potential with minimum side effects.

EXPERIMENTAL

General Notes:

Melting points were determined on Gallenkamp melting point apparatus and are corrected. Ultra violet (U.V.) spectra were measured on a Pye-Unicam SP-800 G spectrophotometer. Infrared (I.R.) spectra were measured in chloroform on a JASCO A-302 spectrometer. Nuclear Magnetic Resonance (NMR) spectra were recorded on Bruker AM 300, 400 and 500 spectrometers operating at 300, 400 and 500 MHz respectively. The chemical shifts are reported in δ (ppm) and coupling constants in Hz. Mass Spectra (MS) were determined using a Finnigan (Varian MAT) 112 or Finnigan MAT-312 double focusing mass spectrometers connected to MAT-188 data system with PDP 11/34 DEC computer system.

Detection of Compounds

Purity of products were checked on TLC plates coated with silica gel and spots were viewed with ultra violet light at 254 nm for fluorescence

quenching spots and at 366 nm for fluorescent spots. Plates were also visualised by spraying iodine vapours.

General Method for the Synthesis of the Derivatives

All the derivatives were synthesised by simple quaternization reaction of the two reactants resulting in formation of the quaternary salts which were collected and purified.

Equimolar quantities of 1-methyl-4hydroxy piperidine and substituted phenacyl halides were dissolved in acetone in separate conical flasks. These were then mixed together in a round bottom flask and refluxed on the water bath until the completion of the reaction.

The reaction was monitored by TLS using a solvent system of $CHCl_3$-MeOH in different combinations. The reaction time for the synthesis of most of the derivatives was 6-8 hours.

After the completion of the reaction or when enough product was formed, the resulting solid material or precipitate was filtered and washed with cold acetone to remove traces of the unreacted starting materials. The collected precipitate or the solid material was then recrystallised with an appropriate solvent.

Each of the synthesised and purified derivative was then analysed for physical properties and its structure was confirmed using the spectroscopic techniques.

The physical properties of the synthesised derivatives are presented in table

CHARACTERIZATION OF COMPOUNDS

1-Methyl-(4'-Methoxyphenacyl)-4-Hydroxypiperidinium Bromide (1)

^{1}H-NMR (CD_3OD, 400 MHz) δ: 8.01 (3H, d, J = 7.31 Hz, H-2', 6'), 7.07 (2H, d, J = 7.31 Hz, H-3', 5'), 6.28 (2H, s, H-α), 3.90 (3H, s, Ar-OCH_3), 3.28 (4H, m, H-2, 6), 2.14 (1H, m, H-4) and 1.91 (4H, m, H- 3, 5).

EIMS *m/z* (relative int., %): 264 (M^+-HBr, $C_{15}H_{22}NO_3$, 6), 150 (1), 120 (2), 114 (100), 100 (8), 92 (2), 82 (13), 80 (14), 77 (3) and 56 (2).

IRν_{max} (KBr) cm^{-1}: 3300, 2900, 1660, 1585, 1250, 1160 and 820.

UVλ_{max} (MeOH) nm: 284, 222 and 202.

1-Methyl-(4'-Bromophenacyl)-4-Hydroxypiperidinium Bromide (2)

^{1}H-NMR (CD_3OD, 400 MHz) δ: 7.94 (2H, d, J = 8.80 Hz, H-2', 6'), 7.76 (2H, d, J = 8.80 Hz, H-3', 5'), 6.12 (2H, s, H-α), 3.09 (4H, m, H-2, 6), 2.14 (1H, m, H-4) and 1.73 (4H, m, H-3, 5).

EIMS *m/z* (relative int., %): 314 (M^+-Br, $C_{14}H_{20}BrNO_2$, 2), 183 (2), 114 (100), 82 (14), 80 (15), 70 (3) and 56 (3).

IRν_{max} (KBr) cm^{-1}: 3350, 2900, 1680, 1570, 1390, 1060 and 800.

UVλ_{max} (MeOH) nm: 261, 203 and 194.

1-Methyl-(3',4'-Dihydroxyphenacyl-4-Hydroxypiperidinium Chloride (3)

^{1}H-NMR (CD_3OD, 400 MHz) δ: 7.45 (1H, dd, J = 2.18 Hz, H-6'), 7.43 (1H, d, J = 2.18 Hz, H-2'), 6.87 (1H, d, J = 8.82 Hz, H-5'), 6.16 (2H, s, H-α), 3.29 (4H, m, H-2, 6), 2.11 (1H, m, H-4) and 1.90 (4H, m, H-3, 5).

EIMS *m/z* (relative int., %): 266 (M^+-HCl, $C_{14}H_{20}NO_4$, 1), 137 (4), 123 (2), 114 (100), 100 (8), 84 (3), 81 (2) and 56 (7).

IRν_{max} (KBr) cm^{-1}: 3440, 2950, 1665, 1590, 1300, 1200 and 810.

UVλ_{max} (MeOH) nm: 234, 222 and 208.

1-Methyl-(3'-Methoxyphenacyl)-4-Hydroxypiperidinium Bromide (4)

^{1}H-NMR (CD_3OD, 400 MHz) δ: 7.63 (1H, ddd, J = 7.70, 2.50, 1.80 Hz, H-6'), 7.52 (1H, dd, J = 2.50, 1.65 Hz, H-2'), 7.47 (1H, t, J = 8.05 Hz, H-5'), 7.25 (1H, ddd, J = 8.05, 2.50, 1.80 Hz, H-4'), 6.24 (2H, s, H-α), 3.86 (3H, s, Ar-OCH_3), 3.12 (4H, m, H-2, 6), 2.14 (1H, m, H-4) and 1.91 (4H, m, H-3, 5).

EIMS *m/z* (relative int., %): 264 (M^+-HBr, $C_{15}H_{22}NO_3$), 135 (39), 114 (100), 107 (26), 105 (1), 92 (15), 86 (12), 82 (10), 77 (26) and 56 (11).

IRν_{max}: ($CHCl_3$) cm^{-1}: 3650, 3300, 1720, 1690, 1590, 1230 and 985.

UVλ_{max} (MeOH) nm: 254, 220 and 201.

1-Methyl-(4'-Nitro)-4-Hydroxypiperidinium Bromide (5)

^{1}H-NMR (CD_3OD, 400 MHz) δ: 8.01 (2H, d, J = 7.31 Hz, H-2', 6'), 7.07 (2H, d, J = 7.31 Hz, H-3', 5'), 6.28 (2H, s, H-α), 3.90 (3H, s, Ar-OCH_3), 3.28 (4H, m,H-2, 6), 2.14 (1H, m, H-4) and 1.91 (4H, m, H-3, 5).

EIMS *m/z* (relative int. %): 310 (M^+-HBr, $C_{15}H_{22}NO_3$, 6), 150 (1), 120 (2), 114 (100), 100 (8), 92 (2), 82 (13), 80 (14), 77 (3) and 56 (2).

IRν_{max} (KBr) cm^{-1}: 3300, 2900, 1660, 1585, 1250, 1160 and 820.

UVλ_{max} (MeOH) nm: 284, 222 and 202.

1-Methyl-(2',4'-Dihydroxy)-4-Hydroxypiperidinium Bromide (6)

^{1}H-NMR (CD_3OD, 400 MHz) δ: 7.80 (1H, d, J = 8.18 Hz, H-6'), 6.60 (1H, dd, J = 8.18, 2.28 Hz, H-5'), 6.58 (1H, d, J = 2.28 Hz, H-3'), 6.25 (2H, s, H-α), 3.21 (4H, m, H-2, 6), 2.72 (1H, m, H-4) and 1.81 (4H, m, H-3, 5).

EIMS *m/z* (relative. int. %) 266 (M^+-HBr, $C_{14}H_{20}NO_4$), 137 (4), 123 (2), 114 (100), 100 (8), 84 (3), 81 (2) and 56 (7).

IRν_{max} (KBr) cm^{-1}: 3440, 2950, 1665, 1590, 1300, 1200 and 810.

UVλ_{max} (MeOH) nm: 234, 222 and 208.

1-Methyl-(4'-Chlorophenacyl)-4-Hydroxypiperidinium Bromide (7)

^{1}H-NMR (CD_3OD, 400 MHz) δ: 8.02 (2H, d, J = 8.83 Hz, H-2', 6'), 7.60 (2H, d, J = 8.83 Hz, H-3', 5'), 6.39 (2H, s, H-α), 3.36 (4H, m, H-2, 6), 2.15 (1H, m, H-4) and 1.81 (4H, m, H-3, 5).

EIMS *m/z* (relative int., %): 269 (M^+-Br, $C_{14}H_{20}ClNO_2$, 1) 139 (13), 114 (100), 101 (2), 84 (3), 82 (11) and 57 (6).

IRν_{max} (KBr) cm^{-1}: 3300, 2900, 1675, 1565, 1250, 1045 and 820.

UVλ_{max} (MeOH) nm: 256, 230 and 203.

1-Methyl-(4'-Fluorophenacyl)-4-Hydroxypiperidinium Chloride (8)

^{1}H-NMR (CD_3OD, 400 MHz) δ: 8.11 (2H, dd, J = 9.03, 5.28 Hz, H-2', 6'), 7.32 (2H, t, J = 9.03 Hz, H- 3', 5'), 6.18 (2H, s, H-α), 3.22 (4H, m, H-2, 6), 2.15 (1H, m, H-4) and 1.90 (4H, m, H-3, 5).

EIMS *m/z* (relative int., %): 252 (M^+-HCl, $C_{14}H_{19}FNO_2$, 1), 123 (7), 114 (100), 101 (15), 100 (6), 96 (2), 84 (6), 82 (3) and 68 (18).

IRν_{max} (KBr) cm^{-1}: 3300, 2900, 1675, 1580, 1230, 1050 and 840.

UVλ_{max} (MeOH) nm: 250, 204 and 192.

1-(4'-Methylphenacyl)-4-Hydroxypiperidinium Bromide (9)

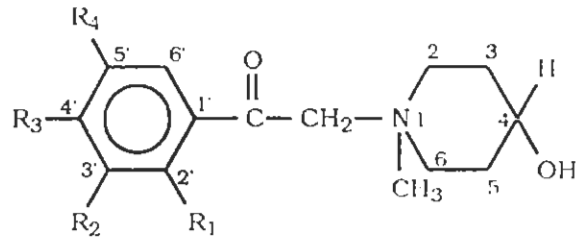
^{1}H-NMR (CD_3OD, 400 MHz) δ: 7.94 (2H, d, J = 8.30 Hz, H-2', 6'), 7.36 (2H, d, J = 8.30 Hz, H-3', 5'), 6.02 (2H, s, H-α), 3.12 (3H, m, H-2, 6), 2.43 (3H, s, Ar-CH_3), 2.05 (1H, m, H-4) and 1.76 (4H, m, H- 3, 5).

EIMS *m/z* (relative int. %): 248 (M^+-HBr $C_{15}H_{22}NO_2$, 1), 135 (19), 120 (2), 114 (100), 105 (1), 91 (25), 84 (3), 76 (1) and 56 (90).

IRν_{max} ($CHCl_3$) cm^{-1}: 3400, 2850, 1655, 1590, 1235, 825.

UVλ_{max} (MeOH) nm: 256, 202 and 193.

Table



Comp.	R_1	R_2	R_3	R_4	Salt	Colour/Shape	M.P. (°C)	Mol. Formula	Yield %
1	H	H	OCH_3	H	HBr	Colourless crystal	165-166	$C_{15}H_{23}BrNO$	64
2	H	H	Br	H	HBr	Colourless rods	260-261	$C_{14}H_{20}Br_2NO_2$	72*
3	H	OH	OH	H	HCl	Ash white crystal	234-235	$C_{14}H_{21}ClNO_4$	78
4	H	OCH_3	H	H	HBr	Yellowish solid	-	$C_{15}H_{23}BrNO_3$	60
5	H	H	NO_2	H	HCl	Colourless crystal	220-221	$C_{14}H_{21}ClNO_4$	60
6	OH	H	OH	H	HCl	White powder	155-158	$C_{14}H_{21}ClNO_4$	65
7	H	H	Cl	H	HBr	Light yellow mass	-	$C_{14}H_{20}BrClNO_2$	65
8	H	H	F	H	HBr	Colourless powder	236-237	$C_{14}H_{20}ClFNO_2$	63
9	H	H	CH_3	H	HBr	Brown gummy	-	$C_{15}H_{23}BrNO_2$	72

PHARMACOLOGICAL STUDIES

EXPERIMENTAL

Neurochemical Estimation

ANIMALS:

Male Albino mice (locally bred), weighing 25-30 gm were caged individually in the same environmental conditions for about four days before experimentation.

DRUGS:

The synthetic compounds were dissolved in the water for injection and injected to the test animals, i.p. at doses of 100 mg/kg, body weight. Pethidine (25 mg/kg) body weight was taken as a standard. Saline was injected to the control animal by the same route.

EXTRACTION PROCEDURE:

Animals were killed 1 hr. after the saline or test compound injection. Brains taken out within one minute were dipped into ice cold saline and were stored at 70°C. Extraction medium was prepared (Haleem *et al.*

1990) by mixing 3.4 ml perchloric acid, 0.1 gm sodium meta Bisulphite ethylene diamine-tetra-acetate 0.001 gm and 0.01 gm cysteine. Volume was made upto 100 ml. Brain samples were homogenized in 5 volumes of extraction medium and centrifuged. The clear supernatant was decanted into eppendrof tubes for storage until analysis.

Brain concentrations of 5-hydroxytryptamine (5-HT), 5-hydroxyindole acetic acid (5-HIAA), dopamine (DA), dihydroxyphenyl acetic acid (DOPAC), homovanillic acid (HVA) and noradrenaline (NA) were determined by HPLC-EC method at 0.8 V electrode potential. A5U shrimp-pack CLC ODS, 4.6 mm ID* 15 cm separation column was used.

The solvent system was methanol (18%), octylsodium sulphate (0.023%) and EDTA (0.0035%) in 0.1 M phosphate buffer (pH = 2.9). Samples from saline, pethidine and test compound injected animals were run in a balanced design using 20 µl loop injector.

Determination of the Analgesic Activity

White Albino mice of either sex (locally bred) weighing between 20-25 gm were used. Analgesic activity of the compound was tested as antinociceptive effect against thermal stimuli (Tail Flick Test).

The animals were maintained in a group of five under standard colony conditions i.e. 12 hrs. light and 12 hrs. dark temperature 21 $\pm$ 2°C fed with balance diet and water.

Pethidine HCl in the dose of 50 mg/kg body weight and all the piperidine derivatives in the same doses i.e. 50 mg/kg were homogenized in 1% aqueous suspension of gum tragacanth. The homogenate including the insoluble fraction was administered orally by incubation of the mice. Maximum liquid given at one time was 0.1 ml.

The method used was a modification of Distasi *et al.* (1988). Mice were held in such a position that the one third of the tail was immersed in

water-bath thermostatically kept at 51°C. The cut off time for immersion was 180 seconds to avoid the injury of tissues of tail.

The reaction time was determined at +30, +60, +90 and +120, +150, +180 and +210 minutes after the administration of the reference and test drugs as post drug tail flick latency. The control animals received only the vehicle..

The mean increase in latency after drug administration or analgesia TFLD ± S.E.M. were calculated.

Statistical analysis were performed using student's t-test and values were considered significant if $P < 0.05$.

Determination of Cytotoxic Activity

A shallow rectangular perforated plastic pot was taken and it contained artificial sea water (3.8% w/v). A partition was made in the rectangular pot with the help of a perforated device but rectangular pot was divided into two unequal compartments.

Brine shrimp (*Artemia salina*) leach eggs were hatched into this pot and then approximately 50 mg of eggs were sprinkled into large compartment which was darkened while the smaller compartment was opened to ordinary light. In this way they were kept about two days and after two days phototropic nauplii were collected by pipette from the lighted side.

Test compound sample was prepared by dissolving 20 mg of each compound in 2 ml of methanol and sets of each of 10, 100 and 1000 µg/ml from this stock solution were transferred to 9 vials, three from each dilution and one vial was kept as control having 2 ml of methanol only. Solvent was allowed to evaporate over night. After two days when shrimp larvae were ready, 1 ml of sea water was added to each vial and

10 shrimps were transferred to each vial (30 shrimps/dilution), and the volume was adjusted with sea water to 5 ml per vial. After 24 hrs. number of survivors were counted. Data were analyzed with Finney computer programme (96) to determine LD_{50}.

Studies on Isolated Intestinal Smooth Muscles Preparation

CONTRACTILE EFFECT

Guinea pig (weighing 500 g) was killed by cervical dislocation, the abdomen opened and a piece of ileum 2 cm was cleaned and suspended in the tissue bath containing tyrode solution aerated with carbogen and maintained at 37°C. Following stabilization period, different doses of samples (**I-IX**) were tested. The contractile response were recorded isotonically using bioscience oscillograph (Gilani A.H., et al. 1986).

RELAXANT EFFECT

The method is same as that of contractile activity except rabbit jejunum was used instead of guinia pig ileum.

CALCIUM CHANNEL BLOCKING ACTIVITY

Guinea pig ileum was used for this purpose. The method is similar to that of contractile effect except the tissue was pretreated with KCl (50

mM). The test drugs were injected cumulatively on contraction muscles. Responses were recorded isotonically using bioscience oscillagraph (Gilani, A.H., 1994).

RESULTS
AND
DISCUSSION

NEUROCHEMICAL ACTIVITY

In view of the possible concern that clinically employed phenyl piperidine analgesics might induce neurotoxic actions on deopamine neurons inthe brain, it is proposed to investigate the effects of newly synthesized N-phenacyl (Ross J.B. *et al.* 1986) derivatives of piperidine on brain catecholamine and indolamine metabolism in mice (100 mg/kg body weight) assuming that these derivatives might alter the brain catecholamine and indole amine differently. The results of this study are depicted in Table 1 and 2 with representative Figs. 1-10. The values are presented as mean $\pm$ SD)n = 5) and the difference significance by Newman Keuls test are presented as ** $p<0.01$ from control following by one way ANOVA.

Figs. 1 and 2 show that the administration of most of the derivatives increased brain dopamine (DA) levels with respect to control. Only compound 9 and 7 cause a decrease in DA level, Figs. 3,4,5 and 6 show that the injection of these compounds (1 - 4 , 5 , 6 - 9) enhance the levels of DOPAC and HVA except 4-nitro moiety (5) which cause

decrease in DOPAC level while 3,4-dihydroxy (3) derivative did not alter HVA level in comparison with the control.

The enhancement of both DA and its metabolites DOPAC and HVA following the administration of most of the derivatives in this series were observed in the present study suggest increase turnover of DA. An increase in the activity of particularly rate limiting catecholamine synthesizing enzyme tyrosine hydroxylase (Sved A.F. and Ferustrom, J.D. 1981) can explain the findings.

An acute deficiency of DA produced by 4-chloro (7) and 4-methyl (9) derivatives led to the conclusion that these may have neurotoxic effect particularly on dopaminergic neurons. However an increase in particularly DOPAC concentration by compounds VII and 9 associated increase in HVA by the same compound suggest that the observed decrease in DA level following compound 9 administration may have occurred because of an acute increase in the release of monoamines.

Administration of compound (8) the para fluoro moiety produce greater increase in the brain DA levels than other compounds of the series. This may possibly occur because of DA antagonistic activity. The present study suggest that compound (8) may have greater neuroleptic like activity, somewhat similarly administration of neuroleptic increase DA metabolism and particularly HVA level (Okeeffe, R. 1970).

Figs. 7 and 8 show that the metabolism of 5-hydroxytraptamine (5HT) were increased significantly followed by the administration of compound (1 - 4 , 6 - 7 and 9). Only the administration of compound 5 and 8 cause decrease in brain 5HT levels.

Figs. 9 and 10 show that 5HIAA level increase by the effect of these newly synthesized derivatives however only compound 8 , did not alter 5-HT level.

A role of indole amine in the antinociceptive effects of morphine is often described in animal studies (Samanin, *et al.* 1978). The present study shows that administration of piperidine increase brain serotonin

metabolism this may occur because of an increase in the availability of tryptophan to the brain or due to an increase in the activity of tryptophan hydroxylase (Schaechter, J.D. *et al.* 1990).

The present study suggest that these drugs (1- 9) may be carefully used in psychotic patients because of their effects on the enhancement of both serotonin and catecholamine metabolism.

Table-1

Effect of *N*-methyl piperidine derivatives (2, 4, 7 and 9) on catecholamine and indolamine levels (mg/g) in mice brain 1 hour after the injection. Values are mean ± S.D (n = 5). Difference significant by Newmann Keuels. Tests were p<0.01, p*<0.05 from control following one way ANOVA.**

Neurotransmitter/ Metabolites	Control Water for Injection	2	4	7	9	ANOVA Diff. 4, 25 F	P
DA	130 ± 18	164 ± 62	203 ± 34	110 ± 04	124 ± 04	0.98	p ≤ 0.01
DOPAC	849 ± 30	1005 ± 84	1017 ± 91	124 ± 62	986 ± 76	0.67	p ≤ 0.01
HVA	133 ± 30	186 ± 48	214 ± 64	157 ± 83	199 ± 64	1.37	p < 0.01
5HT	272 ± 43	199 ± 61	214 ± 54	222 ± 80	284 ± 66	1.4	p < 0.01
5HIAA	314 ± 36	252 ± 48	376 ± 55	364 ± 32	269 ± 66	0.59	p < 0.01

Table-2

Effect of *N*-methyl piperidine derivatives (1 3 ,5 , 6 and 8) on catecholamine and indolamine levels (mg/g) in mice brain 1 hour after the injection. Values are mean ± S.D (n = 5). Difference significant by Newmann Keuels. Tests were p<0.01, p*<0.05 from control following one way ANOVA.**

Neurotransmitter/ Metabolites	Control Water for Injection	1	3	5	6	8	ANOVA Diff. 4, 25	
							F	P
DA	119 ± 38	140 ± 64	225 ± 32	406 ± 80	338 ± 41	818 ± 43	0.78	p < 0.01
DOPAC	192 ± 62	241 ± 72	350 ± 24	135 ± 78	603 ± 45	513 ± 73	1.3	p ≤ 0.01
HVA	73 ± 38	141 ± 15	70 ± 26	111 ± 21	190 ± 45	117 ± 11	0.82	p < 0.01
5HT	88 ±22	16 ± 07	36 ± 18	42 ± 13	202 ± 36	59 ± 19	1.3	p < 0.01
5HIAA	117 ± 39	28.5 ± 08	86 ± 32	390 ± 38	78 ± 30	157 ± 42	0.36	p < 0.01

Fig. 1: The effects of N-methylphenacyl derivatives of piperidine (**1**, **3**, **5**, **6**, and **8**) on mice brain DA levels.

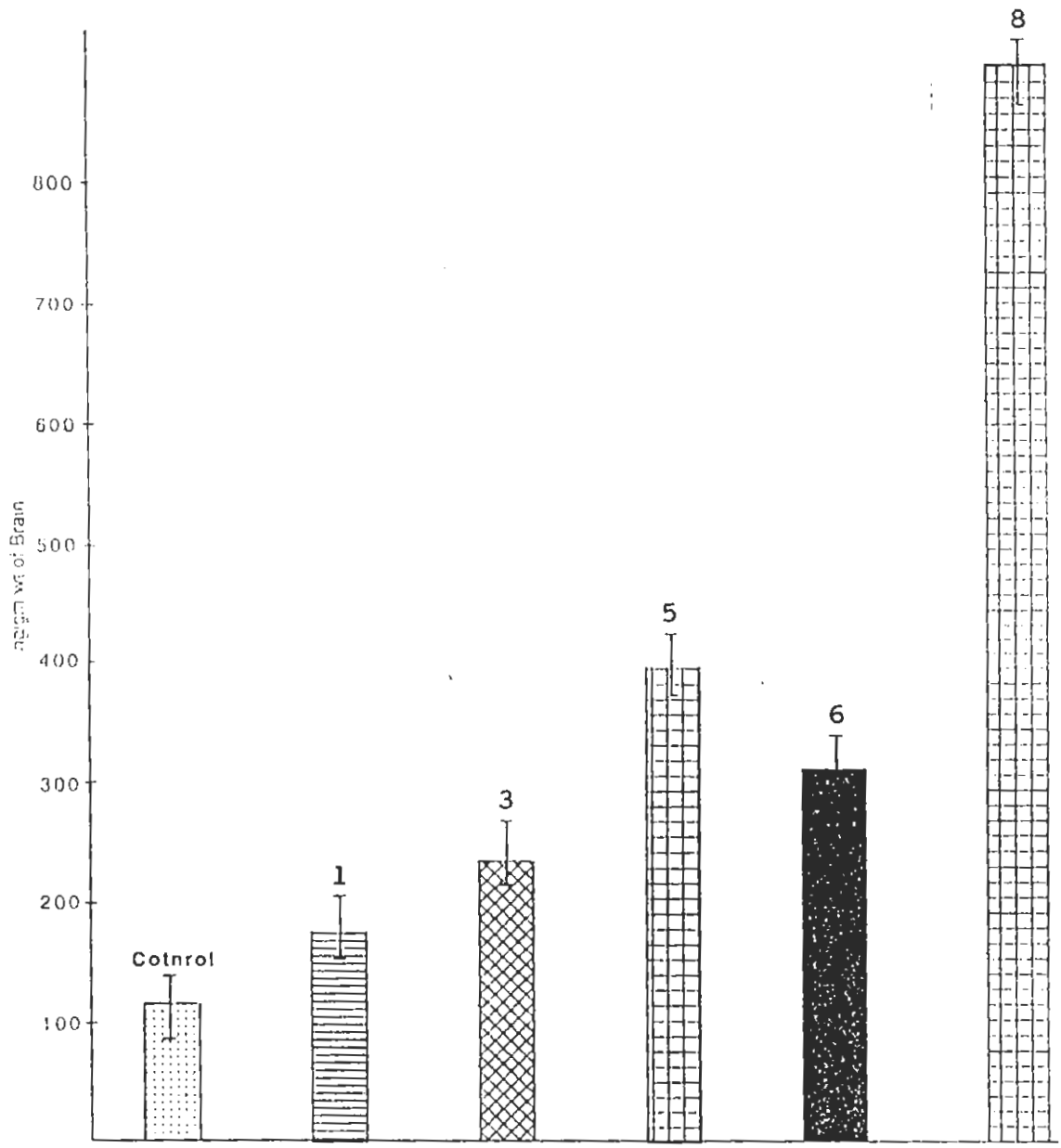


Fig. 2: The effects of N-methylphenacyl derivatives of piperidine (**2**, **4**, **7** and **9**) on mice brain DA levels.

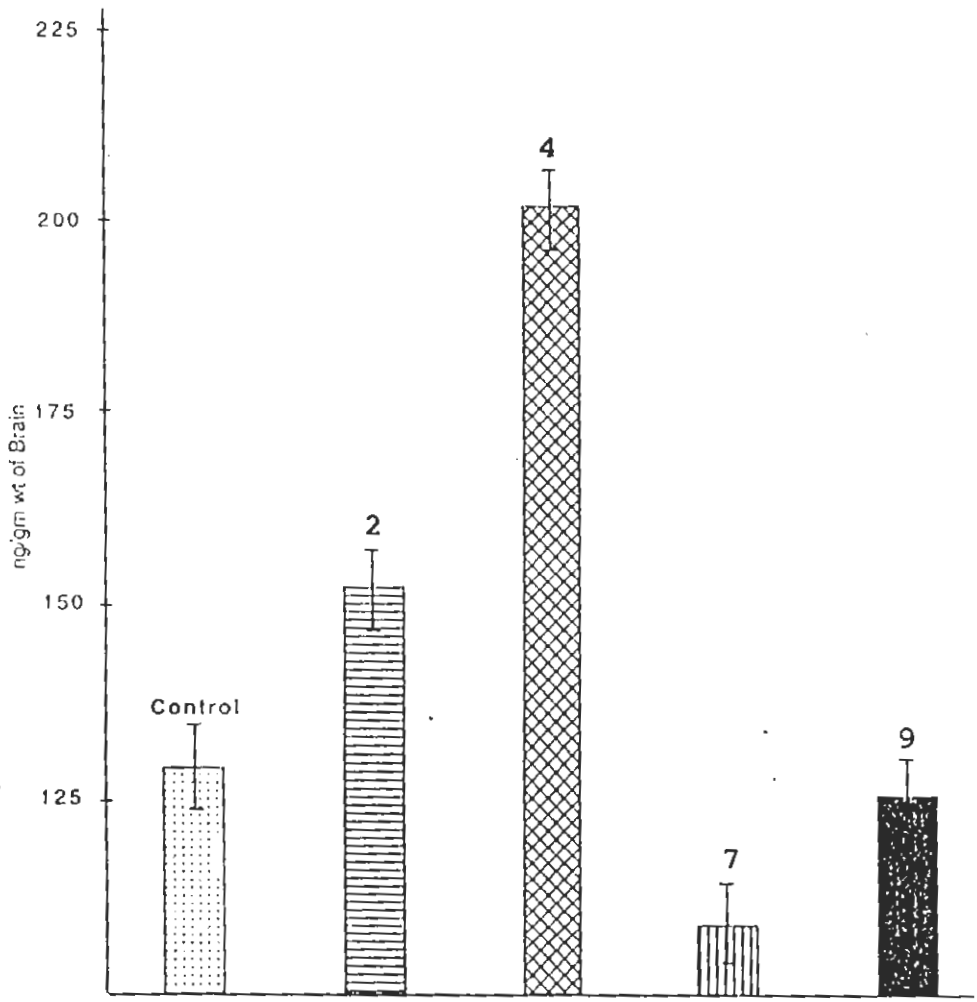


Fig. 3: The effects of N-methylphenacyl derivatives of piperidine (1, 3, V, 6, and **8**) on mice brain DOPAC levels.

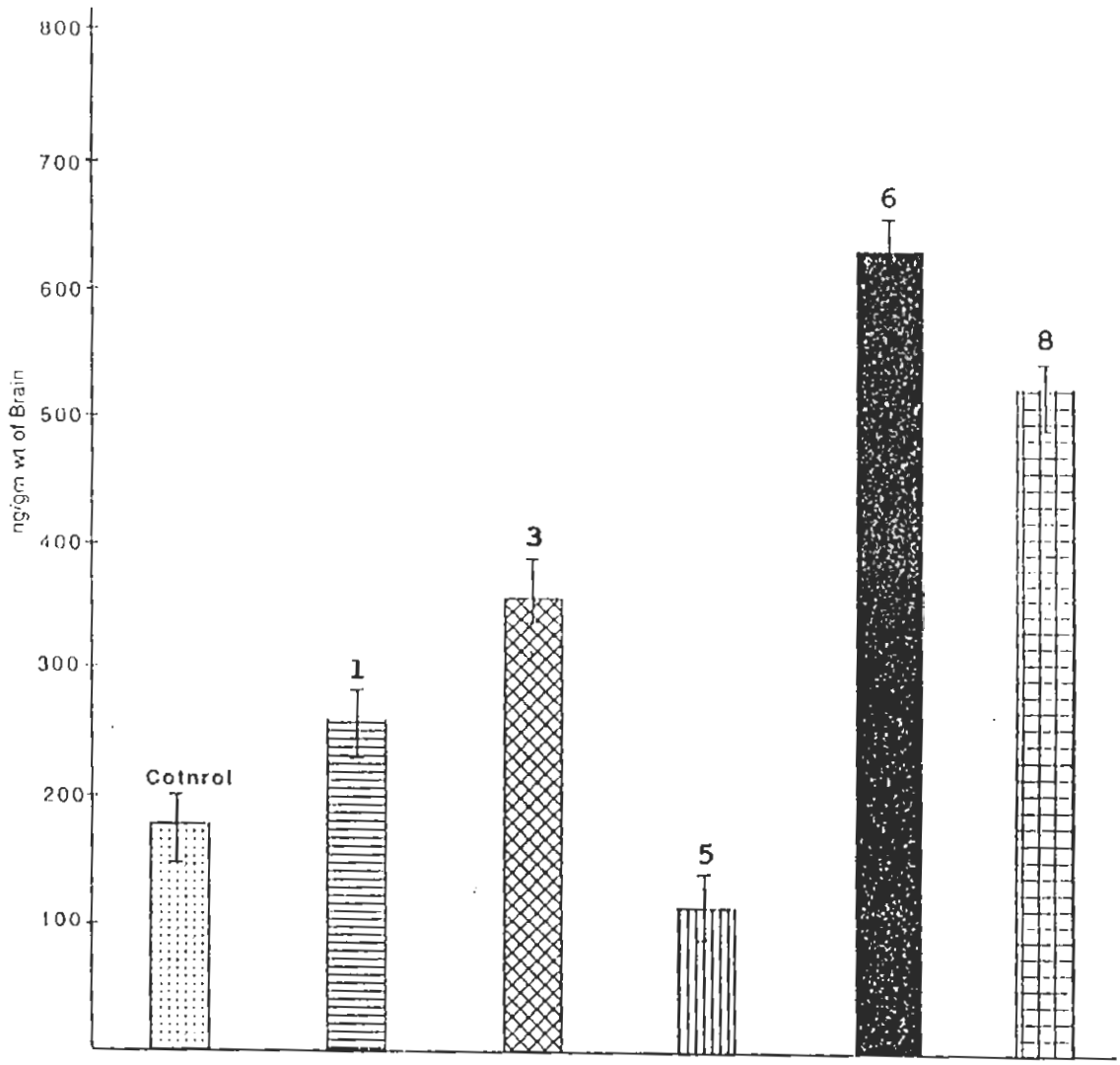


Fig. 4: The effects of N-methylphenacyl derivatives of piperidine (II, **4**, 7 and **9**) on mice brain DOPAC levels.

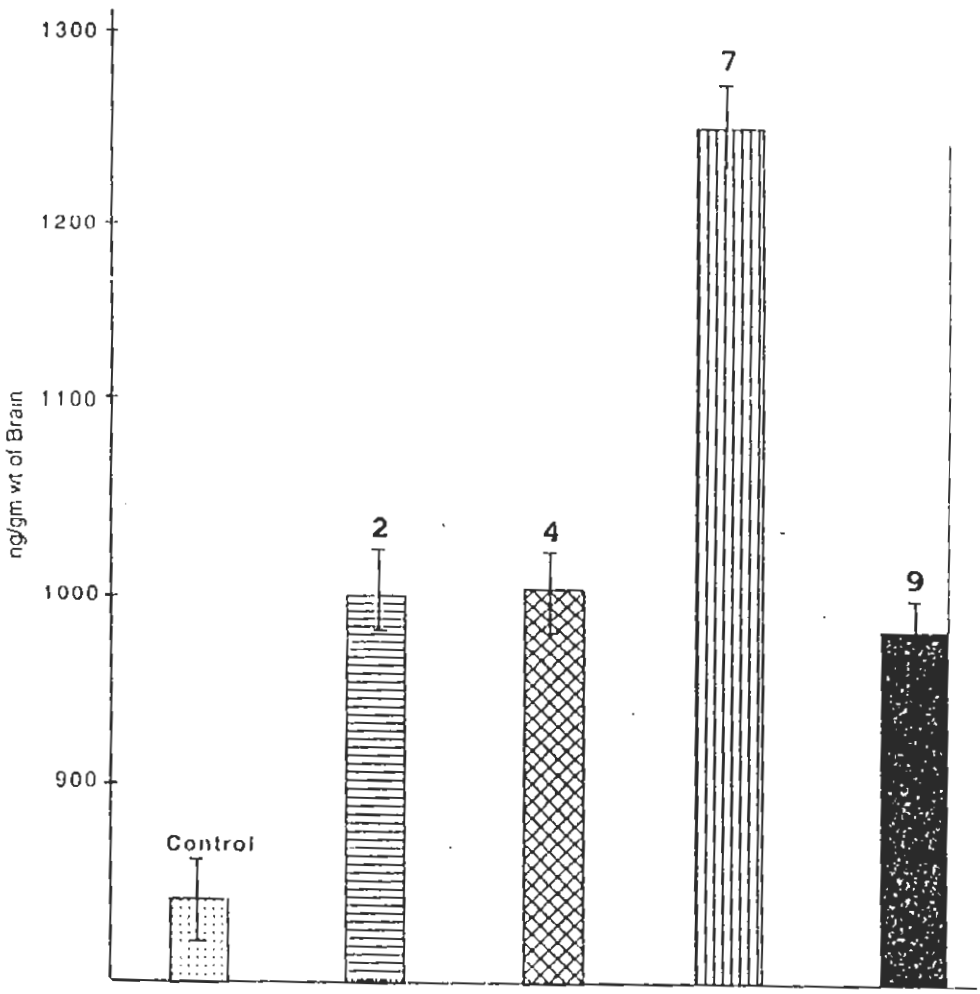


Fig. 5: The effects of N-methylphenacyl derivatives of piperidine (**1**, **3**, **5**, **6**, and **8**) on mice brain HVA levels.

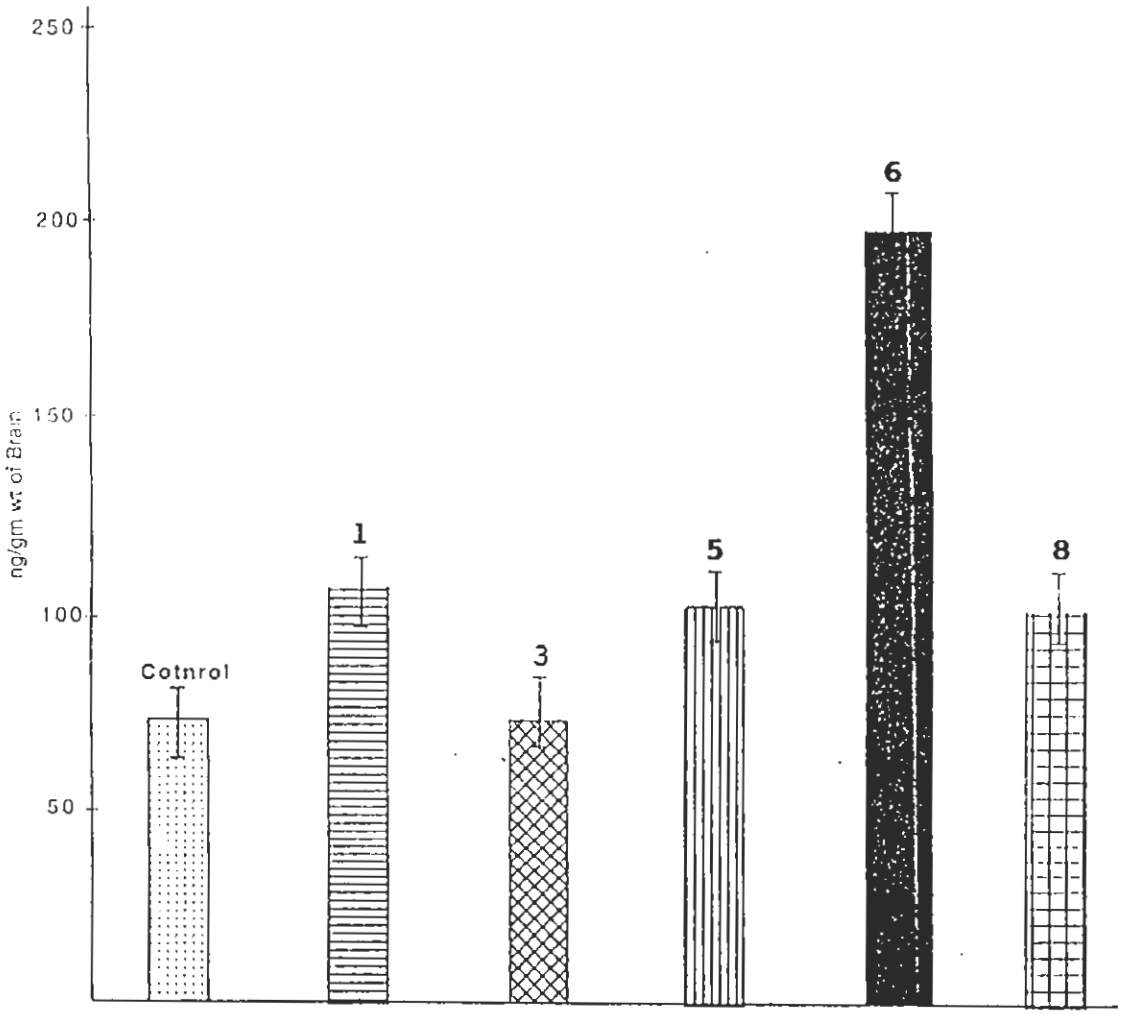


Fig. 6: The effects of N-methylphenacyl derivatives of piperidine (II, 4, 7 and 9) on mice brain HVA levels.

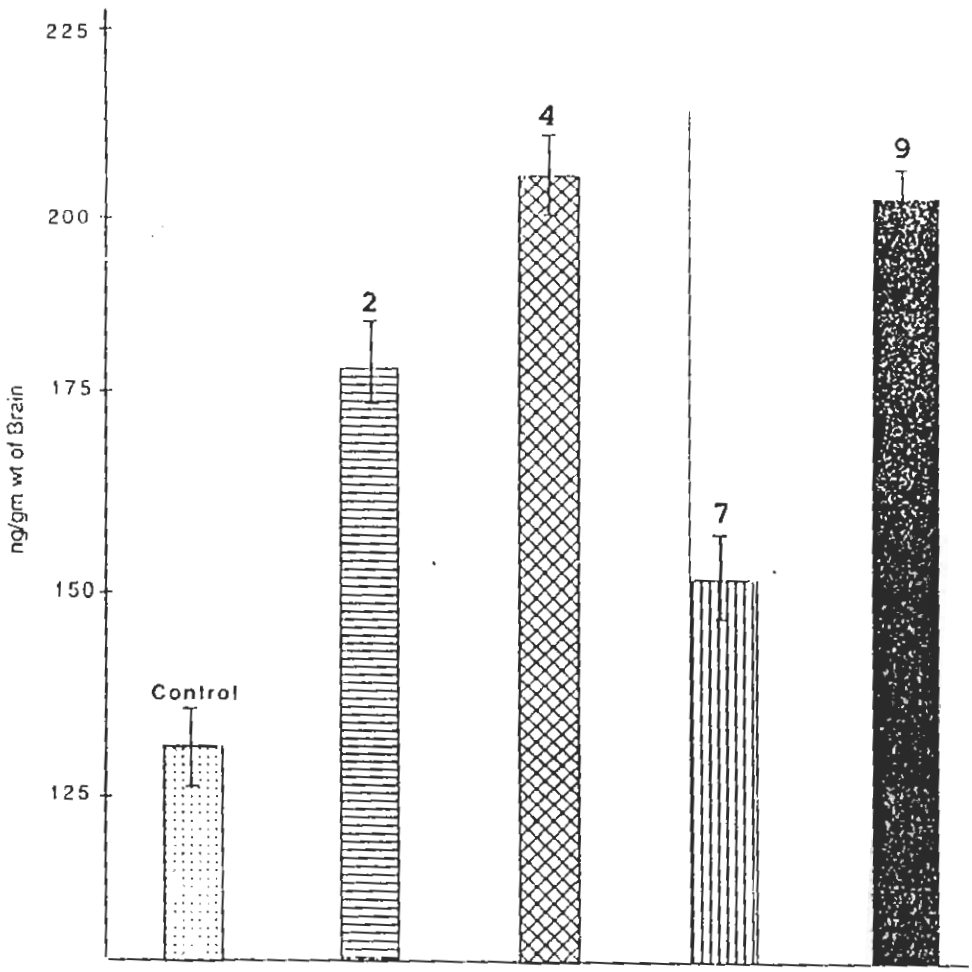


Fig. 7: The effects of N-methylphenacyl derivatives of piperidine (1 3, 5, 6, and 8) on mice brain 5HT levels.

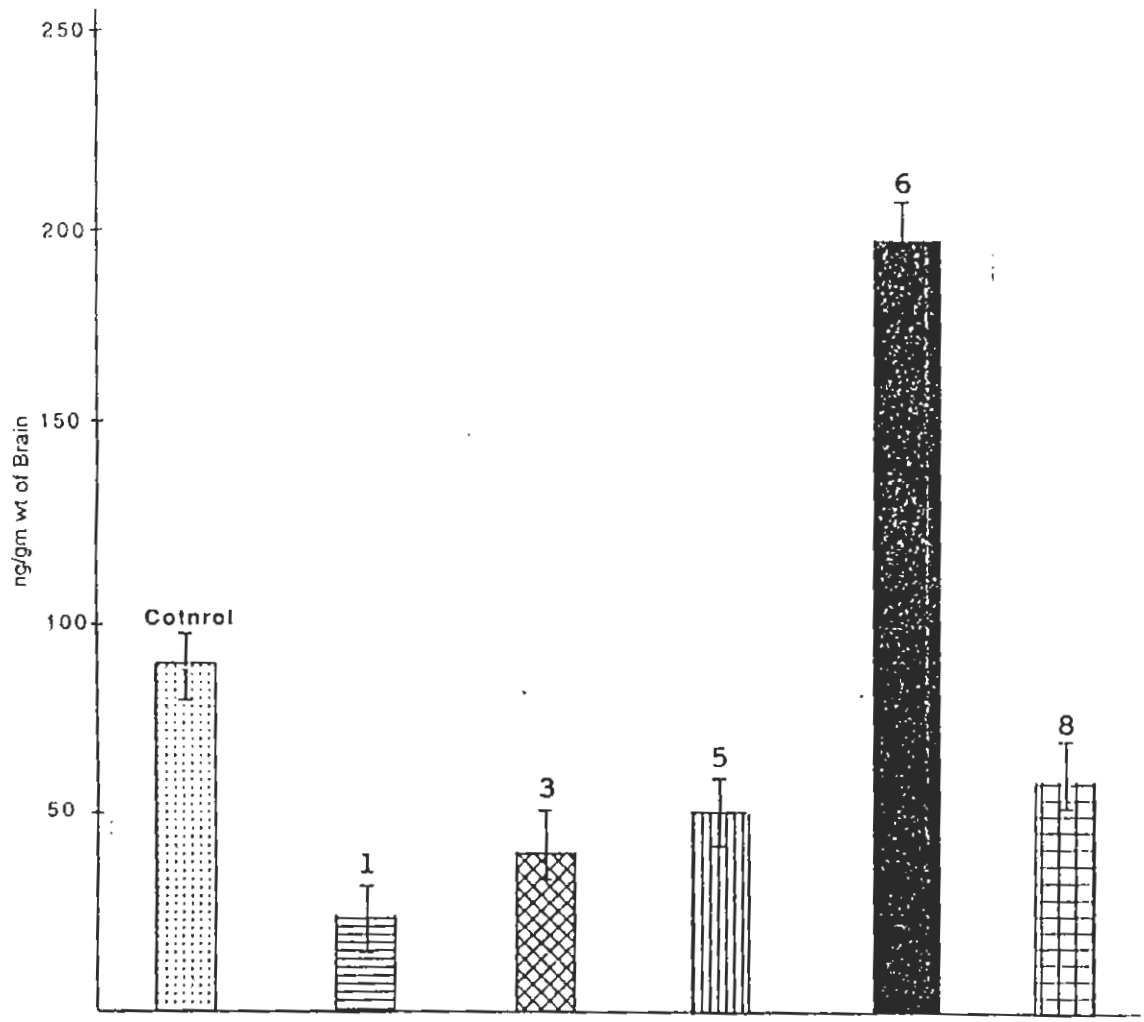


Fig. 8: The effects of N-methylphenacyl derivatives of piperidine (2, 4 , 7 and 9) on mice brain 5HT levels.

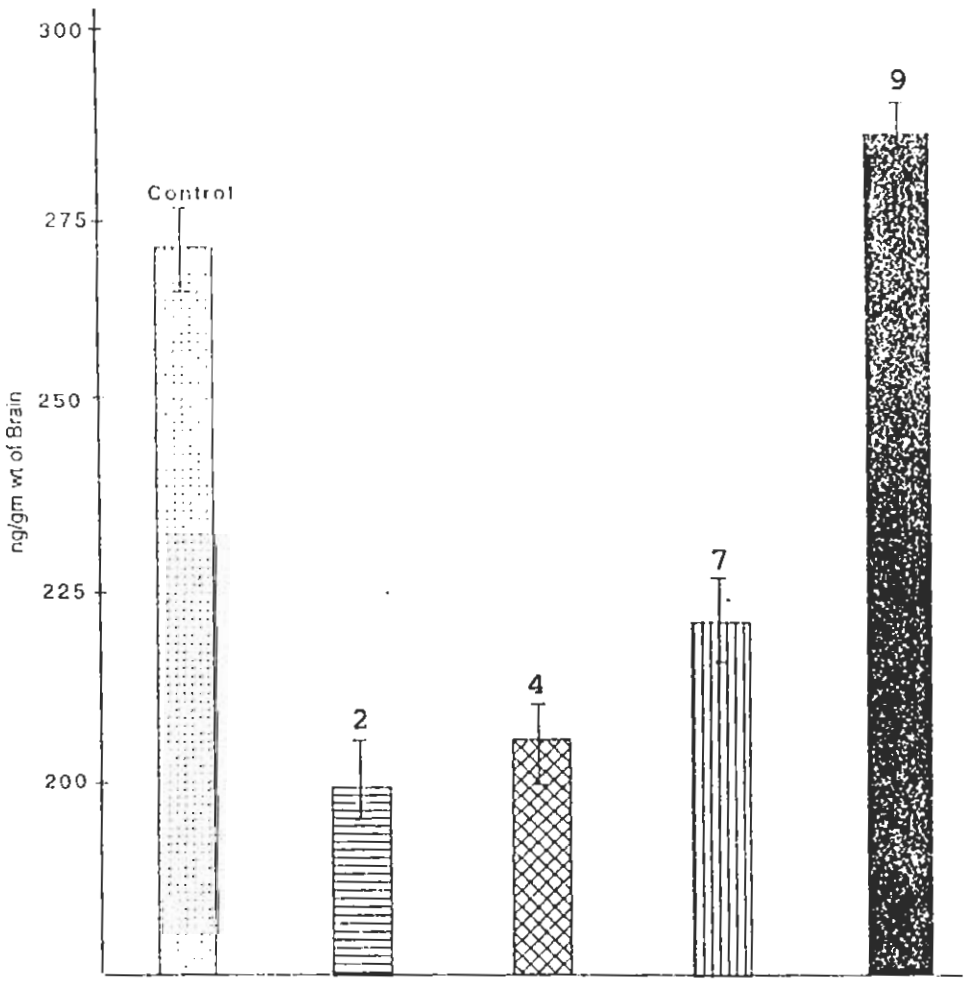
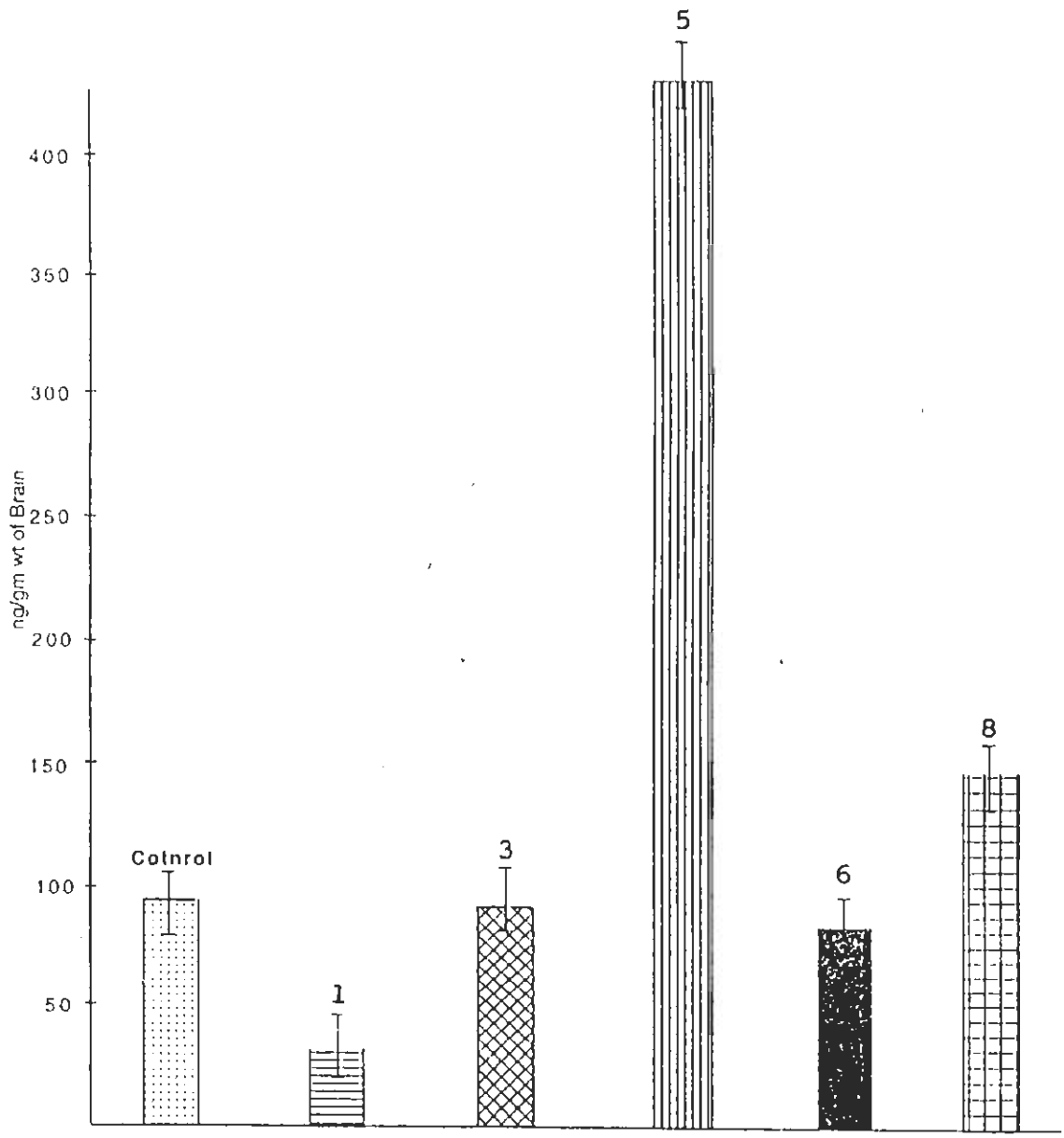
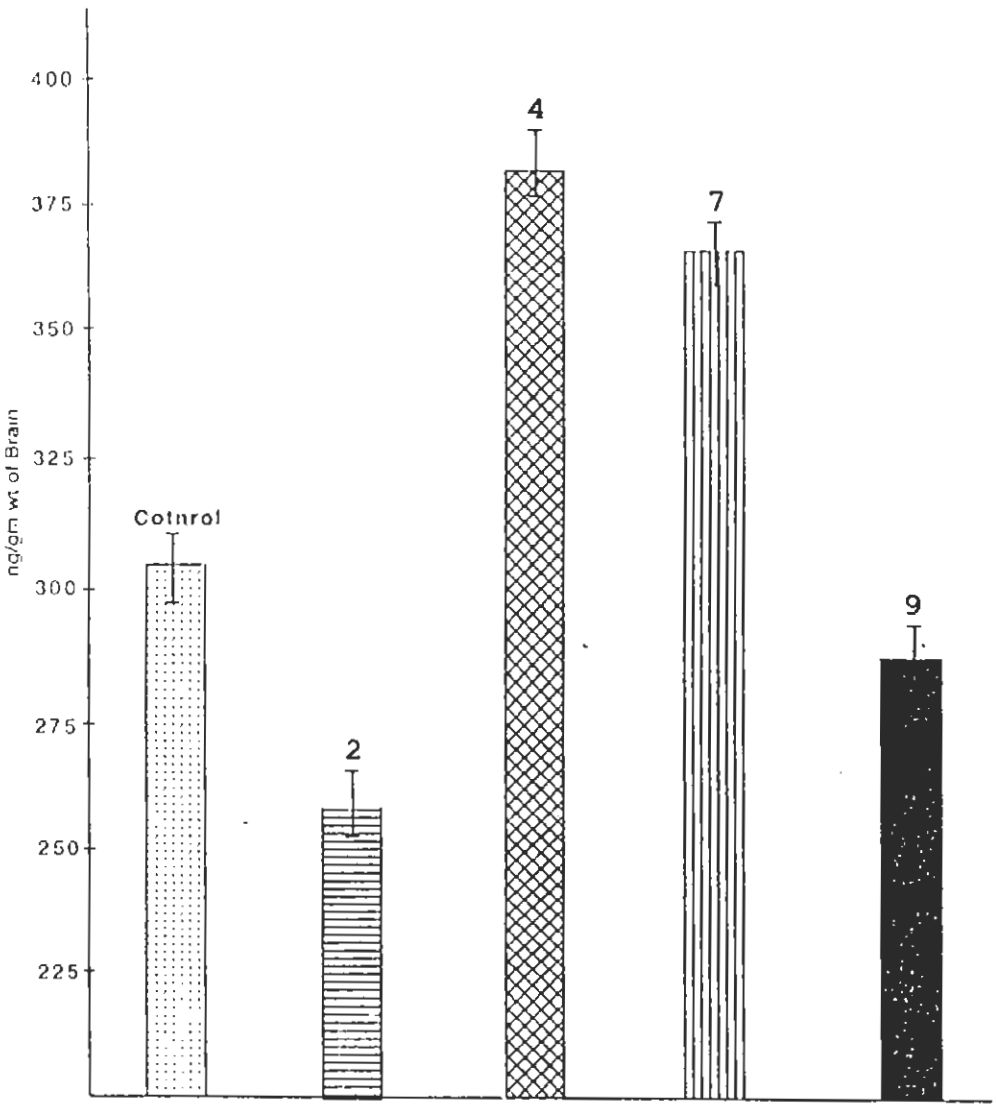


Fig. 9: The effects of N-methylphenacyl derivatives of piperidine (1 3 , 5 , 6 , and 8) on mice brain 5HIAA levels.



0: The effects of N-methylphenacyl derivatives of piperidine (2, 4 , 7 and 9) on mice brain 5HIAA levels.



ANALGESIC ACTIVITY

The results of the analgesic activity of newly synthesized piperidinium derivatives recorded are summarized in Tables 3-11 and also presented in Fis. 11-19. The results are indicated as "analgesia TFLD" (Tail Flick Latency Difference) or "mean increase in latency after drug administration ± SEM" in terms of second(s). Figs. 11-19 illustrate the values of "analgesia TFLD to time intervals". Results show that all the piperidinium derivatives possessed varying degrees of analgesic activity tested at a particular dose. The results of the individual compounds are presented in this section.

1-Methyl-1 (4-Fluro) Phenacyl-4-Hydroxy Piperidinium Bromide (8)

The analgesic activity of this compound is shown in Table-10 and represented in Fig. 18. It showed varying degree of analgesic activity in the dose of 50 mg/kg. This compound showed highly significant ($p < 0.01$) analgesic effects after +30, +60, +90, +120, +150, +180 and +210 minutes. The maximum analgesia TFLD value was achieved at +120 minutes i.e. 3.40 ± 0.32. While pethidine HCl (50 mg/kg) induced highly significant analgesic effect ($p < 0.01$) at +120 and +150 minutes as 1.72 ± 0.23 and 1.52 ± 0.23 respectively.

1-Methyl-1 (3-Methoxy) Phenacyl-4-Hydroxy Piperidinium Bromide (4)

The analgesic effects of 1-methyl-1 (3-methoxy) phenacyl-4-hydroxy piperidinium bromide are summarized in Table-6 and represented by Fig. 14. The compound showed a highly significant analgesic activity ($p < 0.01$) at the dose level of 50 mg/kg after +30, +60, +90, +120 and +150 minutes. The analgesic effect induced by this compound was peaked at +60 minutes (1.84 ± 0.21) whereas at +180 and +210 minutes it showed the significant analgesic response ($p<0.05$) and showed analgesia TFLD values of 1.48 ± 0.30 and 1.60 ± 0.40 respectively.

1-Methyl-1 (4-Nitro) Phenacyl-4-Hydroxy Piperidinium Bromide (5)

The analgesic activity of this compound is summarized in Table-7 and represented in Fig. 15. It showed a varying degree of analgesic response. The results indicate that this compound showed a highly significant A($p < 0.01$) activity at the time interval of 30, 60 and 90 minutes with analgesia TFLD values of 1.60 ± 0.26, 2.04 ± 0.38, 2.04 ± 0.29 and the significant analgesic response ($p < 0.05$) was achieved at +120 and +150 minutes at the analgesia TFLD values of 1.76 ± 0.33 and 1.48 ± 0.33 respectively. After 150 minutes the analgesic activity was diminished.

1-Methyl (4-Methoxy) Phenacyl-4-Hydroxy Piperidinium Bromide (1)

The compound 1-methyl (4-methoxy) phenacyl-4-hydroxy piperidinium bromide possessed a varying degree of analgesic activity tested at the dose level of 50 mg/kg. The results of this compound were summarized in Table-3 and Fig. 11. It showed a highly significant ($p<0.01$) analgesic activity after +30, +60, +90, +120 and +150 minutes with analgesia TFLD values of 2.08 ± 0.20, 2.48 ± 0.38, 2.72 ± 0.24, 2.44 ± 0.90, 1.76 ± 0.22. The significant ($p < 0.05$) analgesic response was observed after +180 and +210 minutes with analgesia TFLD values of 1.72 ± 0.39 and 1.40 ± 0.30 respectively.

1-Methyl-1 (4-methyl) Phenacyl-4-Hydroxy Piperidinium Bromide (9)

In this study, this compound showed a significant analgesic activity at the dose of 50 mg/kg. The results of analgesic response of 1-methyl-1 (4-methyl) phenacyl-4-hydroxy piperidinium bromide are summarized in Table-11 and Fig. 19. It showed analgesia TFLD which is quite comparable to that of reference drug. The significant ($p<0.05$) analgesic activity was observed after +30 minute of its administration, then the highly significant ($p<0.01$) analgesic activity was observed after +60 minute as 1.96 ± 0.34 analgesia TFLD values. After +90, +120 and +150 minutes again it showed the significant ($p < 0.05$) analgesic effect with

analgesia TFLD values of 1.84 ± 0.33, 1.64 ± 0.39 and 1.52 ± 0.34 respectively. The activity was diminished after +180 minutes.

1-Methyl-1 (4'-Bromo) Phenacyl-4-Hydroxypiperidinium Bromide (2)

The analgesic activity of this compound was summarized in Table-4 and represented in Fig. 12. It possessed the highly significant but little bit different analgesic activity tested at the dose of 50 mg/kg, and its analgesia TFLD was quite comparable to that of reference drug pethidine HCl.

1-Methyl-1 (3,4-Dihydroxy) Phenacyl-4-Hydroxy Piperidinium Chloride (3)

The results of analgesic activity of 1-methyl-1 (3,4-dihydroxy) phenacyl-4-hydroxy piperidinium chloride and pethidine HCl (the reference drug) were summarized in Table-5 and represented in Fig. 13. In the dose of 50 mg/kg it possessed a highly significant analgesic activity relative to control group. In terms of "analgesia TFLD" the analgesic effects at this dose are expressed as 0.92 ± 0.04 ($p < 0.05$), 1.52 ± 0.12 ($p < 0.01$), 2.04 ± 0.23 ($p < 0.01$), 2.32 ± 0.57 ($p < 0.01$), 1.52 ± 0.27 ($p < 0.05$) at the time intervals of +30, +60, +90, +120 and +150 respectively. After +150 minutes its analgesic effect was lost.

1-Methyl-1 (4-Chloro) Phenacyl-4-Hydroxy Piperidinium Bromide (7)

This compound showed a little bit different type of analgesic activity at the dose of 50 mg/kg. The results are summarized in Table-9 and represented in Fig. 17. It shows highly significant analgesic activity at +30 minutes after that no activity was observed at least for one hours. Again the highly significant activity was observed after +120 minutes after that the activity was diminished. Mean increase in latency values at +30 and +120 minutes are expressed as 1.30 ± 0.06 ($p < 0.01$) and $1.40 + 0.38$ ($p < 0.01$).

1-Methyl-1 (2',4'-Difluro) Phenacyl-4-Hydroxy Piperidinium Chloride (6)

The results of analgesic effect of pethidine HCl (as reference drug) and 1-methyl-1 (2',4'-difluro) phenacyl-4-hydroxy piperidinium chloride at the dose level of 50 mg/kg recorded, are summarized in Table-8 and represented in Fig. 16. The activity was expressed as "mean increase in latency after drug administration" relative to the controls receiving 1% gum tragacanth solution in a dose of 0.1 ml, orally. In the dose level of 50 mg/kg this compound showed the values of "mean increase in latency after drug administration" as 1.40 ± 0.38 ($p < 0.05$), 1.70 ± 0.19 ($p < 0.05$) seconds at +60, +90, +120 and +150 minutes time intervals, respectively.

PETHIDINE HCl (THE REFERENCE DRUG)

Pethidine HCl is a potent analgesic, belongs to the group of opioid analgesic. in the present study it is used as a reference drug for comparison purpose. The results of analgesic activity of all the test piperidinium derivatives were compared with the analgesic activity of pethidine HCl. At the dose level of 50 mg/kg pethidine HCl showed the values of "mean increase in latency" are 1.28 $\pm$ 0.39 ($p < 0.05$), 1.56 $\pm$ 0.34 ($p < 0.05$), 1.56 $\pm$ 0.34 ($p < 0.05$), 1.72 $\pm$ 0.23 ($p < 0.01$), 1.52 $\pm$ 0.23 ($p < 0.01$) seconds at the time interval of +30, +60, +90, +120 and +150 minutes, respectively.

The highly significant ($p < 0.01$) analgesic response was observed +30, +60, +90 and +120 minutes as items increase in latency after administration values of 1.84 $\pm$ 0.35, 2.56 $\pm$ 0.31, 2.64 $\pm$ 0.32 and 2.43 $\pm$ 0.34 respectively. After 50 minute the significant ($p < 0.05$) analgesic response was observed as 2.56 $\pm$ 0.44, it showed a highly significant ($p < 0.01$) analgesic activity after + 180 and +210 minutes as ($p < 0.05$) latency after any administration values of 2.40 + 2.32 and ($p < 0.01$) respectively,

Table-3

Analgesic effect of 1-methyl-1 (4P-methoxy) phenacyl-4-hydroxy piperidinium bromide in mouse tail immersion method

Treatment	Dosage/kg	Analgesic TFLD or mean increase in latency after drug administration ± SEM (s)						
	Orally	+30	+60	+90	+120	+150	+180	+210
Gum tragacanth	50 mg	0.28 ± 0.13	0.60 ± 0.16	0.80 ± 0.22	0.68 ± 0.21	0.72 ± 0.23	0.88 ± 0.10	0.52 ± 0.17
Pethidine HCl	50 mg	1.28 ± 0.39*	1.56 ± 0.34*	1.56 ± 0.34*	1.72 ± 0.23**	1.52 ± 0.23**	1.40 ± 0.29	1.36 ± 0.48
1-methyl-1 (4P-methoxy) phenacyl-4-hydroxy piperidinium bromide	50 mg	2.08 ± 0.20**	2.48 ± 0.38**	2.72 ± 0.24**	2.44 ± 0.09*	1.76 ± 0.22**	1.72 ± 0.39*	1.40 ± 0.30

n/groups = 5
*$p < 0.05$
** $p < 0.01$

Table-4

Analgesic effect of 1-methyl-1 (4-bromo) phenacyl-4-hydroxy piperidinium bromide in mouse tail immersion method

Treatment	Dosage/kg	Analgesic TFLD or mean increase in latency after drug administration ± SEM (s)						
	Orally	+30	+60	+90	+120	+150	+180	+210
Gum tragacanth	50 mg	0.28 ± 0.13	0.60 ± 0.16	0.80 ± 0.22	0.68 ± 0.21	0.72 ± 0.23	0.88 ± 0.10	0.52 ± 0.17
Pethidine HCl	50 mg	1.28 ± 0.39*	1.56 ± 0.34*	1.56 ± 0.34*	1.72 ± 0.23**	1.52 ± 0.23**	1.40 ± 0.29	1.36 ± 0.48
1-methyl-1 (4-bromo) phenacyl-4-hydroxy piperidinium bromide	50 mg	1.84 ± 0.35**	2.56 ± 0.31**	2.64 ± 0.37**	2.48 ± 0.34*	2.56 ± 0.44*	2.40 ± 0.38**	1.84 ± 0.36**

n/groups = 5
*$p < 0.05$
** $p < 0.01$

Table-5

Analgesic effect of 1-methyl-1 (3,4-dihydroxy) phenacyl-4-hydroxy piperidinium chloride in mouse tail immersion method

Treatment	Dosage/kg	Analgesic TFLD or mean increase in latency after drug administration ± SEM (s)						
	Orally	+30	+60	+90	+120	+150	+180	+210
Gum tragacanth	50 mg	0.28 ± 0.13	0.60 ± 0.16	0.80 ± 0.22	0.68 ± 0.21	0.72 ± 0.23	0.88 ± 0.10	0.52 ± 0.17
Pethidine HCl	50 mg	1.28 ± 0.39*	1.56 ± 0.34*	1.56 ± 0.34*	1.72 ± 0.23**	1.52 ± 0.23**	1.40 ± 0.29	1.36 ± 0.48
1-methyl-1 (3,4-dihydroxy) phenacyl-4-hydroxy piperidinium chloride	50 mg	0.92 ± 0.04*	1.52 ± 0.12**	2.04 ± 0.23**	2.32 ± 0.57**	1.52 ± 0.27*	1.04 ± 0.19	1.00 ± 0.00

n/groups = 5
*$p < 0.05$
** $p < 0.01$

Table-6

Analgesic effect of 1-methyl-1 (3-methoxy) phenacyl-4-hydroxy piperidinium bromide in mouse tail immersion method

Treatment	Dosage/kg	Analgesic TFLD or mean increase in latency after drug administration ± SEM (s)						
	Orally	+30	+60	+90	+120	+150	+180	+210
Gum tragacanth	50 mg	0.28 ± 0.13	0.60 ± 0.16	0.80 ± 0.22	0.68 ± 0.21	0.72 ± 0.23	0.88 ± 0.10	0.52 ± 0.17
Pethidine HCl	50 mg	1.28 ± 0.39*	1.56 ± 0.34*	1.56 ± 0.34*	1.72 ± 0.23**	1.52 ± 0.23**	1.40 ± 0.29	1.36 ± 0.48
1-methyl-1 (3-methoxy) phenacyl-4-hydroxy piperidinium bromide	50 mg	1.80 ± 0.16**	1.84 ± 0.21**	1.72 ± 0.27**	1.56 ± 0.25**	1.40 ± 0.20**	1.48 ± 0.30*	1.60 ± 0.40*

n/groups = 5
*$p < 0.05$
** $p < 0.01$

Table-7

Analgesic effect of 1-methyl-1 (4-nitro) phenacyl-4-hydroxy piperidinium bromide in mouse tail immersion method

Treatment	Dosage/kg	Analgesic TFLD or mean increase in latency after drug administration ± SEM (s)						
	Orally	+30	+60	+90	+120	+150	+180	+210
Gum tragacanth	50 mg	0.28 ± 0.13	0.60 ± 0.16	0.80 ± 0.22	0.68 ± 0.21	0.72 ± 0.23	0.88 ± 0.10	0.52 ± 0.17
Pethidine HCl	50 mg	1.28 ± 0.39*	1.56 ± 0.34*	1.56 ± 0.34*	1.72 ± 0.23**	1.52 ± 0.23**	1.40 ± 0.29	1.36 ± 0.48
1-methyl-1 (4-nitro) phenacyl-4-hydroxy piperidinium bromide	50 mg	1.68 ± 0.26**	2.04 ± 0.37**	2.04 ± 0.29**	1.76 ± 0.33*	1.48 ± 0.33*	1.28 ± 0.35	0.72 ± 0.37

n/groups = 5
*p < 0.05
** p < 0.01

Table-8

Analgesic effect of 1-methyl-1 (2',4'-difluro) phenacyl-4-hydroxy piperidinium chloride in mouse tail immersion method

Treatment	Dosage/kg	Analgesic TFLD or mean increase in latency after drug administration ± SEM (s)						
	Orally	+30	+60	+90	+120	+150	+180	+210
Gum tragacanth	50 mg	0.28 ± 0.13	0.60 ± 0.16	0.80 ± 0.22	0.68 ± 0.21	0.72 ± 0.23	0.88 ± 0.10	0.52 ± 0.17
Pethidine HCl	50 mg	1.28 ± 0.39*	1.56 ± 0.34*	1.56 ± 0.34*	1.72 ± 0.23**	1.52 ± 0.23**	1.40 ± 0.29	1.36 ± 0.48
1-methyl-1 (2',4'-difluro) phenacyl-4-hydroxy piperidinium chloride	50 mg	1.00 ± 0.00	1.40 ± 0.38*	1.70 ± 0.19**	1.50 ± 0.06**	1.30 ± 0.19*	1.00 ± 0.12	0.90 ± 0.19

n/groups = 5
*p < 0.05
** p < 0.01

Table-9

Analgesic effect of 1-methyl-1 (4-chloro) phenacyl-4-hydroxy piperidinium bromide in mouse tail immersion method

Treatment	Dosage/kg	Analgesic TFLD or mean increase in latency after drug administration ± SEM (s)						
	Orally	+30	+60	+90	+120	+150	+180	+210
Gum tragacanth	50 mg	0.28 ± 0.13	0.60 ± 0.16	0.80 ± 0.22	0.68 ± 0.21	0.72 ± 0.23	0.88 ± 0.10	0.52 ± 0.17
Pethidine HCl	50 mg	1.28 ± 0.39*	1.56 ± 0.34*	1.56 ± 0.34*	1.72 ± 0.23**	1.52 ± 0.23**	1.40 ± 0.29	1.36 ± 0.48
1-methyl-1 (4-chloro) phenacyl-4-hydroxy piperidinium bromide	50 mg	1.30 ± 0.06*	1.00 ± 0.00	1.30 ± 0.31	1.40 ± 0.38**	1.30 ± 0.31	1.20 ± 0.38	1.10 ± 0.31

n/groups = 5
*p < 0.05
** p < 0.01

Table-10

Analgesic effect of 1-methyl-1 (4-fluro) phenacyl-4-hydroxy piperidinium bromide in mouse tail immersion method

Treatment	Dosage/kg	Analgesic TFLD or mean increase in latency after drug administration ± SEM (s)						
	Orally	+30	+60	+90	+120	+150	+180	+210
Gum tragacanth	50 mg	0.28 ± 0.13	0.60 ± 0.16	0.80 ± 0.22	0.68 ± 0.21	0.72 ± 0.23	0.88 ± 0.10	0.52 ± 0.17
Pethidine HCl	50 mg	1.28 ± 0.39*	1.56 ± 0.34*	1.56 ± 0.34*	1.72 ± 0.23**	1.52 ± 0.23**	1.40 ± 0.29	1.36 ± 0.48
1-methyl-1 (4-fluro) phenacyl-4-hydroxy piperidinium bromide	50 mg	2.20 ± 0.19**	2.97 ± 0.41**	3.16 ± 0.18**	3.40 ± 0.32**	2.80 ± 0.39**	2.56 ± 0.43**	2.16 ± 0.42**

n/groups = 5
*p < 0.05
** p < 0.01

Table-11

Analgesic effect of 1-methyl-1 (4-methyl) phenacyl-4-hydroxy piperidinium bromide in mouse tail immersion method

Treatment	Dosage/kg	Analgesic TFLD or mean increase in latency after drug administration ± SEM (s)						
	Orally	+30	+60	+90	+120	+150	+180	+210
Gum tragacanth	50 mg	0.28 ± 0.13	0.60 ± 0.16	0.80 ± 0.22	0.68 ± 0.21	0.72 ± 0.23	0.88 ± 0.10	0.52 ± 0.17
Pethidine HCl	50 mg	1.28 ± 0.39*	1.56 ± 0.34*	1.56 ± 0.34*	1.72 ± 0.23**	1.52 ± 0.23**	1.40 ± 0.29	1.36 ± 0.48
1-methyl-1 (4-methyl) phenacyl-4-hydroxy piperidinium bromide	50 mg	1.16 ± 0.27*	1.96 ± 0.34**	1.84 ± 0.33*	1.64 ± 0.39*	1.52 ± 0.34*	1.04 ± 0.18	0.92 ± 0.20

n/groups = 5
*$p < 0.05$
** $p < 0.01$

Fig. 11: Analgesic effect of compound 1) in mouse tail immersion method. Results are expressed as analgesia TFLD ± S.E.M.

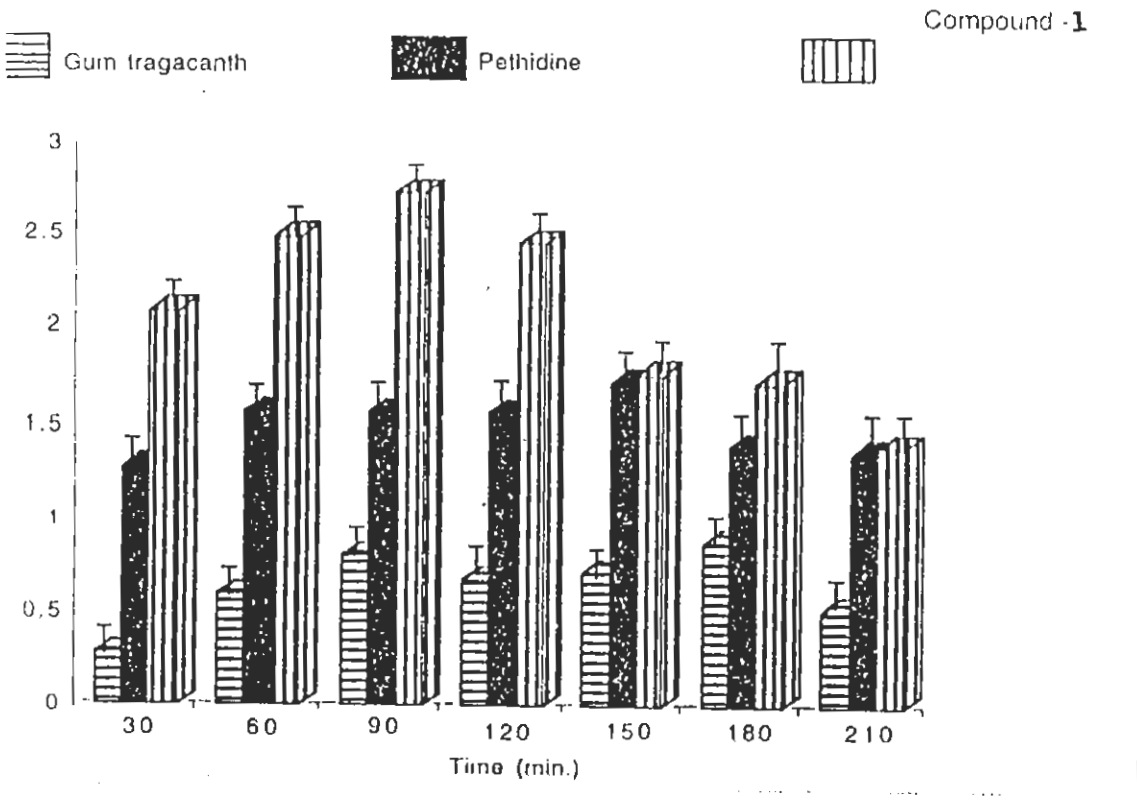


Fig. 12: Analgesic effect of compound (2) in mouse tail immersion method. Results are expressed as analgesia TFLD ± S.E.M.

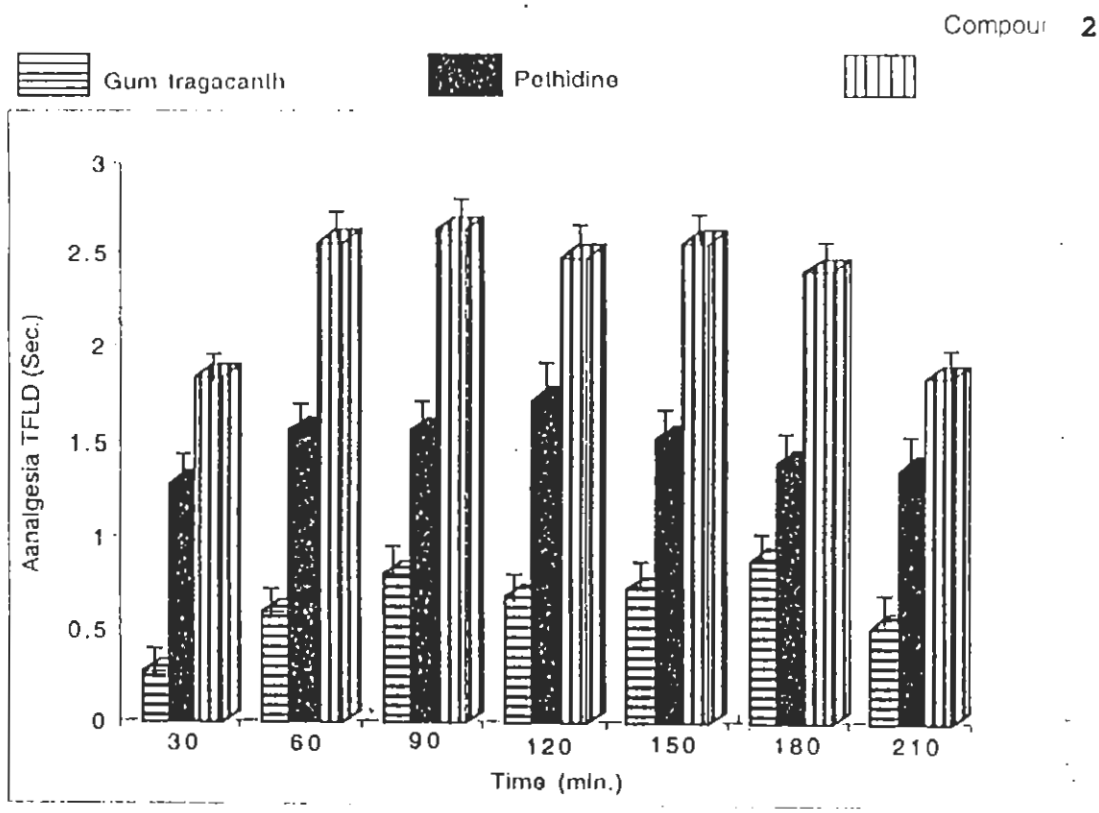


Fig. 13: Analgesic effect of compound (3) in mouse tail immersion method. Results are expressed as analgesia TFLD ± S.E.M.

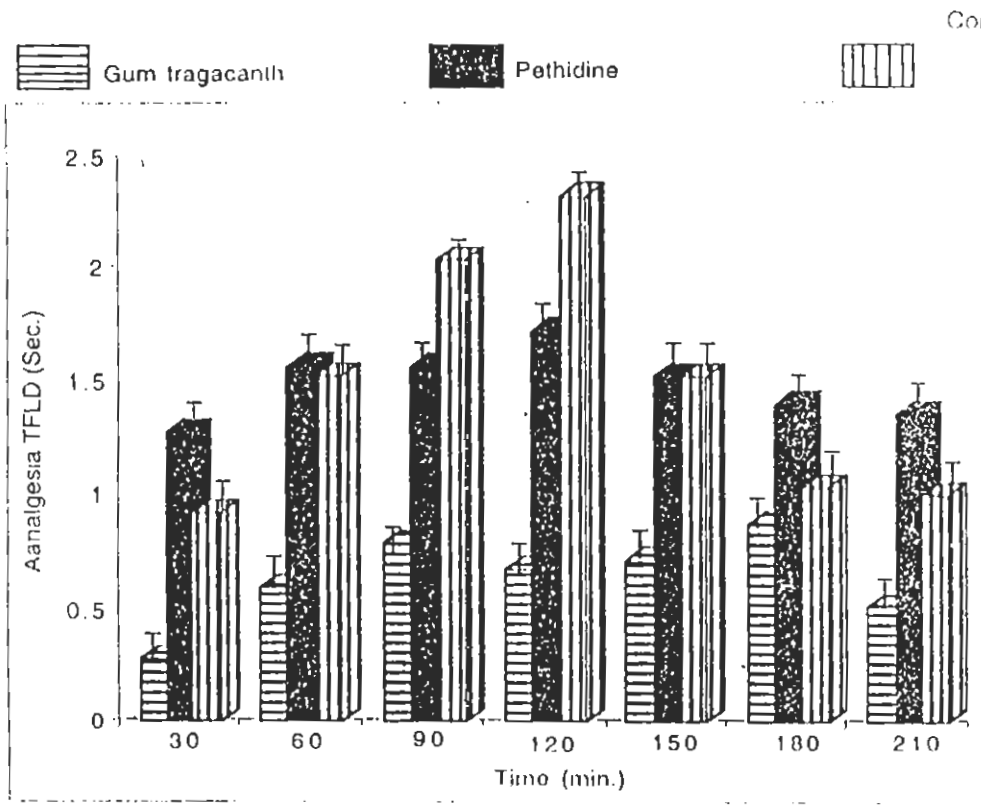


Fig. 14: Analgesic effect of compound (**4**) in mouse tail immersion method. Results are expressed as analgesia TFLD ± S.E.M. (n = 5), $p^* < 0.05$, $p^{**} < 0.01$.

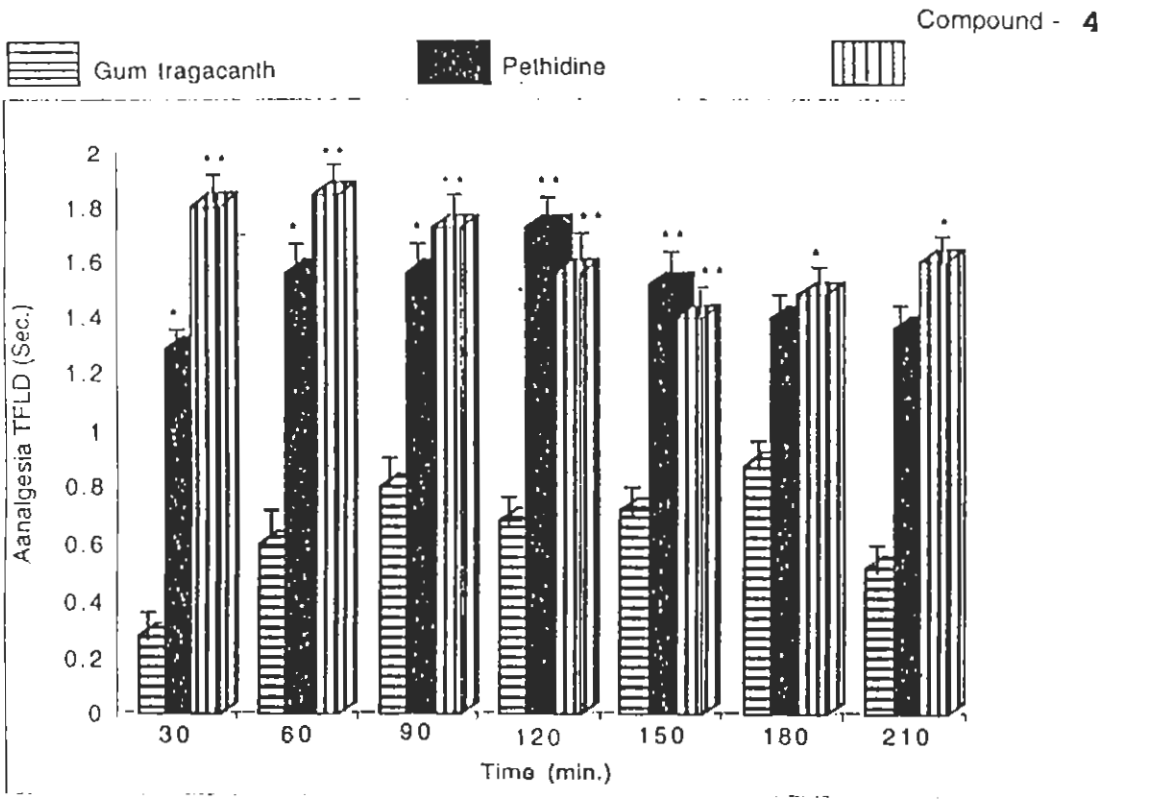


Fig. 15: Analgesic effect of compound (5) in mouse tail immersion method. Results are expressed as analgesia TFLD ± S.E.M.

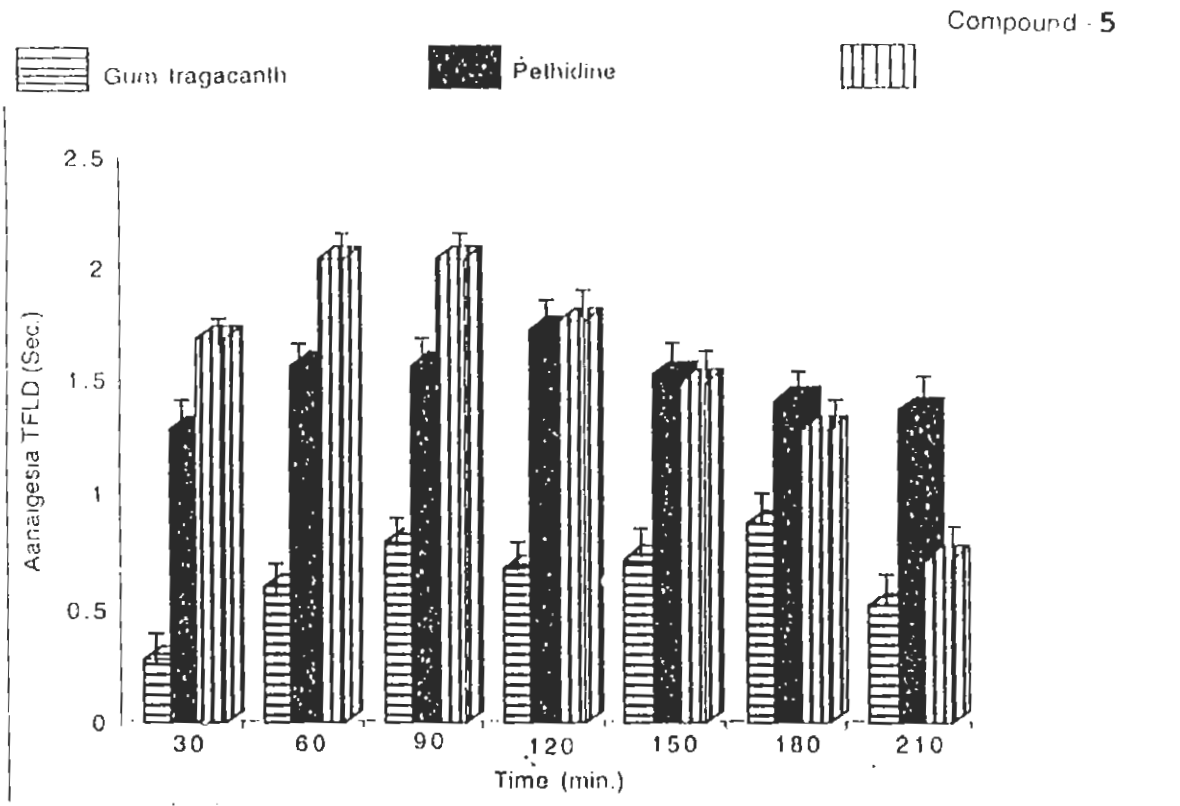


Fig. 16: Analgesic effect of compound (6) in mouse tail immersion method. Results are expressed as analgesia TFLD ± S.E.M.

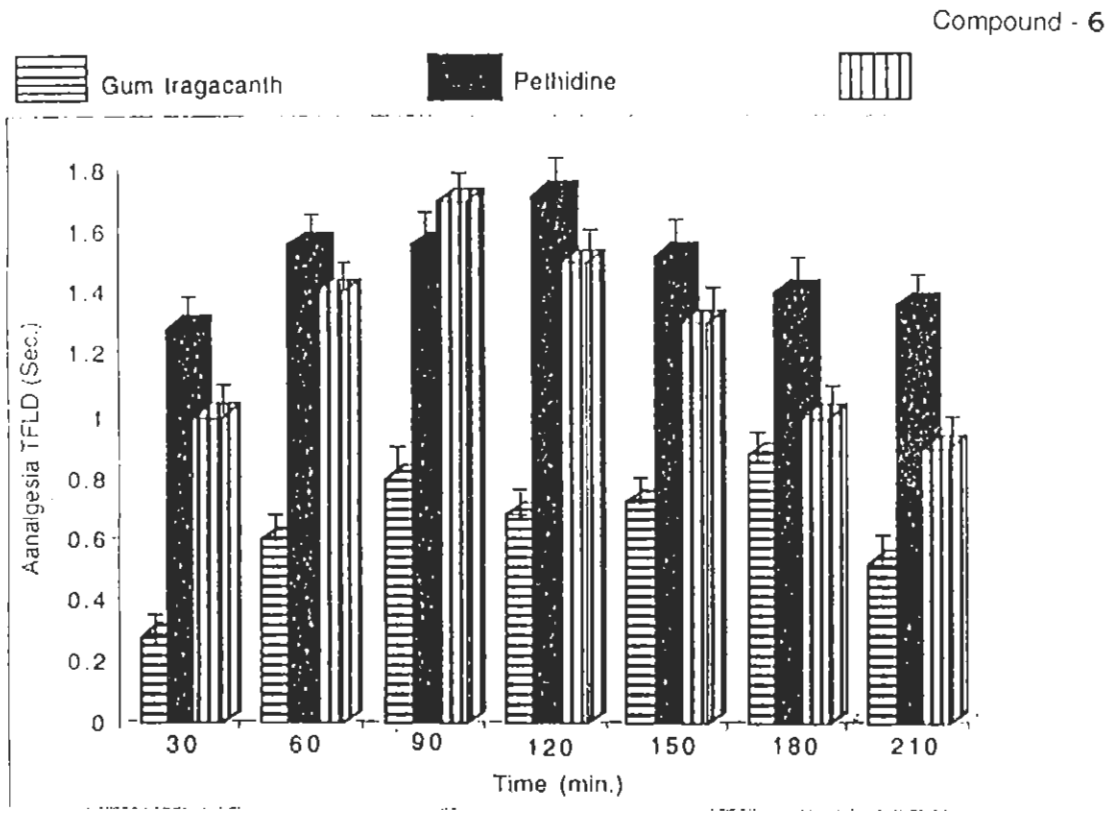


Fig. 17: Analgesic effect of compound (7) in mouse tail immersion method. Results are expressed as analgesia TFLD ± S.E.M.

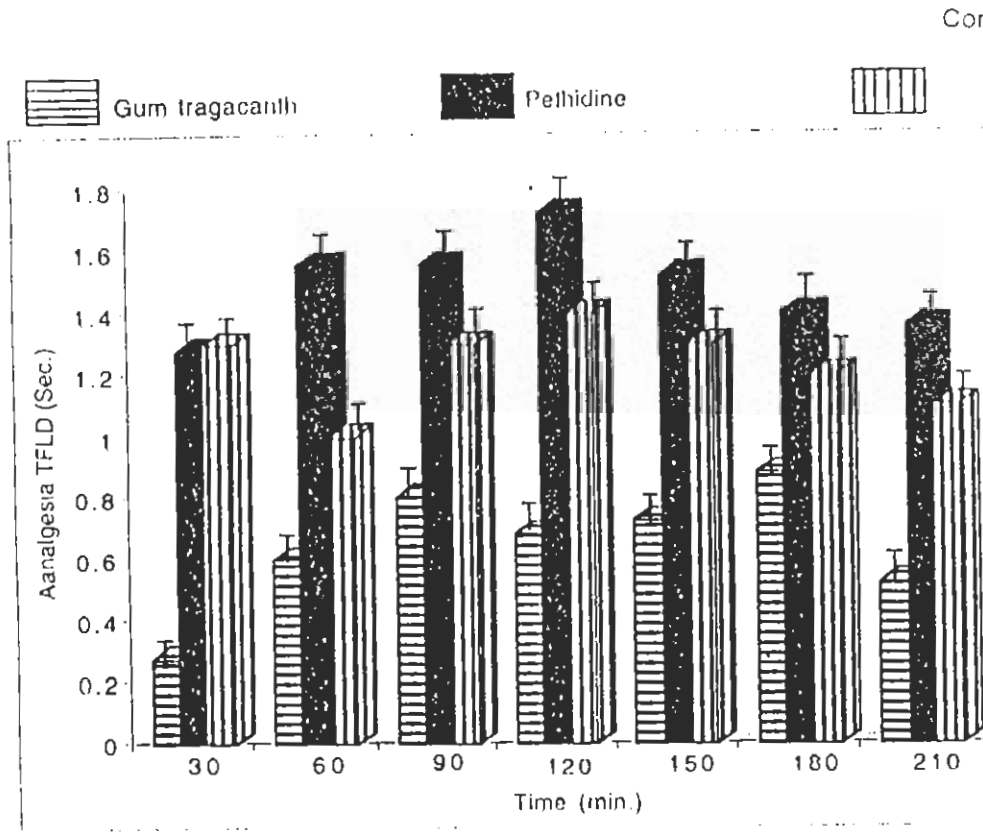


Fig. 18: Analgesic effect of compound (8) in mouse tail immersion method. Results are expressed as analgesia TFLD ± S.E.M. (n = 5), $p^* < 0.05$, $p^{**} < 0.01$.

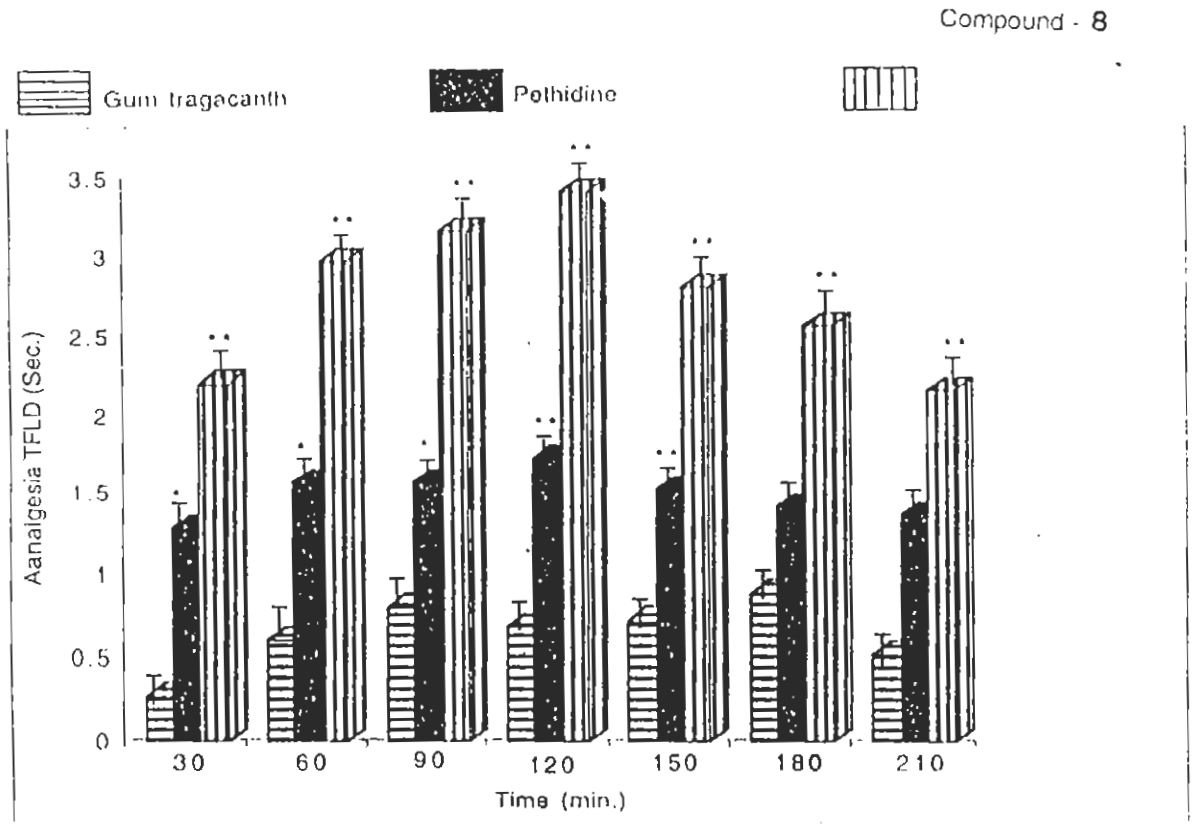
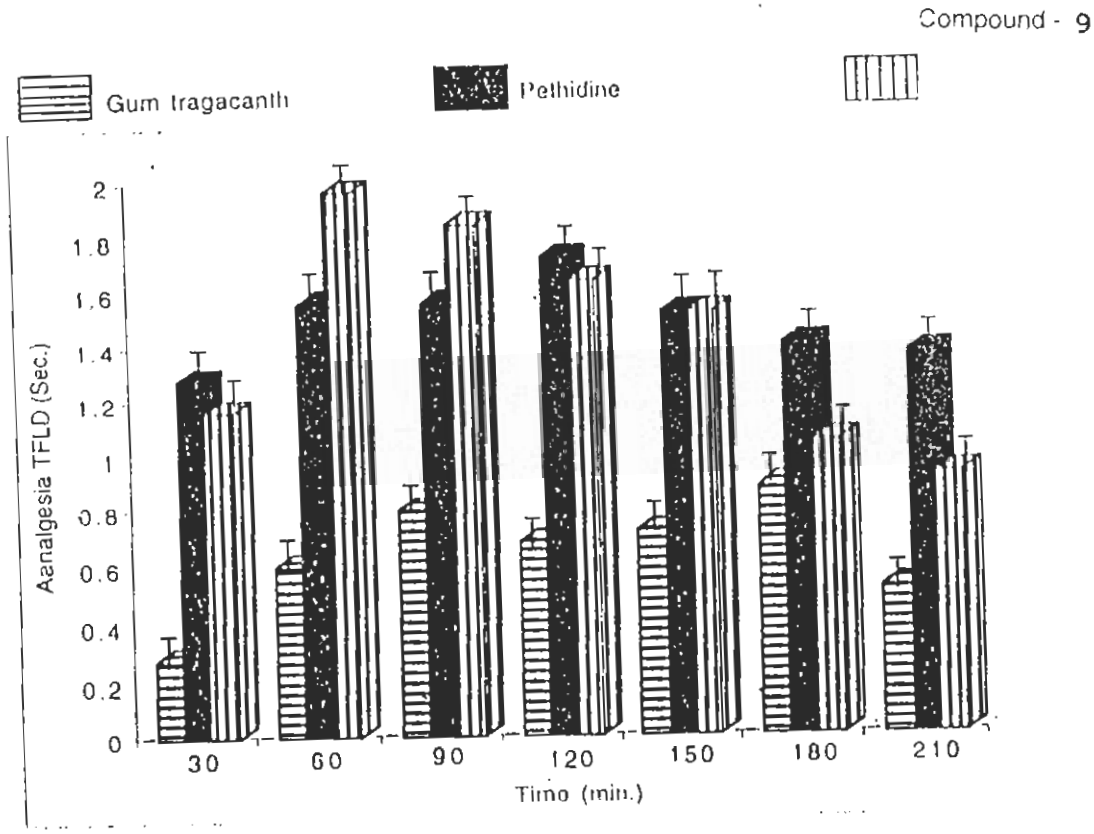


Fig. 19: Analgesic effect of compound (9) in mouse tail immersion method. Results are expressed as analgesia TFLD ± S.E.M.



CYTOTOXICITY

The cytotoxicity of substituted phenacyl derivatives (I - 9.) as well as the parent compound (*N*-methylpiperidine) has been assayed by brine shrimp bioassay using *Artemia salina*. Results in term of LD_{50} in mg/ml are shown in Table-12.

From the data recorded it is evident that the parent compound and most of its derivatives have no considerable cytotoxic activity. Only four derivatives (2, 5, 7 and 8) were found to exhibit lesser degree of toxicity against *Artimia salina* with LD_{50} < 1000 mg/ml. the rest of five compounds (I, 3. 4, 6 and 9) were devoid of cytotoxicity (LD_{50} > 1000).

Regarding the structure activity only monohalogenated derivatives (2 7 and 8) and para nitro moiety (5) showed a low level of cytotoxicity (LD_{50} 886.412, 857.108, 83.636 and 823.238 respectively). suggesting that halogen and nitro group in the phenacyl part of molecule may possibly showed effect on cytotoxicity, however the piperidine part of molecule did not alter the cytotoxicity.

Table-12

Brine shrimp assay results for (I) and its phenacyl derivatives (II-IX)

S. No.	Name of Compound	LD_{50} mg/ml
1.	1-Methyl-1 (4-methoxy) phenacyl-4-hydroxy piperidinium bromide (I)	> 1000
2.	1-Methyl-1 (4'-bromo) phenacyl-4-hydroxy piperidinium bromide (2)	886.142
3.	1-Methyl-1 (3,4-dihydroxy) phenacyl-4-hydroxy piperidinium chloride (3)	> 1000
4.	1-Methyl-1 (3-methoxy) phenacyl-4-hydroxy piperidinium bromide (4)	> 1000
5.	1-Methyl-1 (4-nitro) phenacyl-4-hydroxy piperidinium bromide (5)	823.238
6.	1-Methyl-1 (2,4-difluoro) phenacyl-4-hydroxy piperidinium chloride (6)	> 1000
7.	1-Methyl-1 (4-chloro) phenacyl-4-hydroxy piperidinium bromide (7)	857.108
8.	1-Methyl-1 (4-fluoro) phenacyl-4-hydroxy piperidinium bromide (8)	843.636
9.	1-Methyl-1 (4-methyl) phenacyl-4-hydroxy piperidinium bromide (9)	> 1000

EFFECT ON ISOLATED INTESTINAL SMOOTH MUSCLES

All the compounds (1 - 9) along with the parent compound were evaluated for their effects on isolated intestinal smooth muscles in vivo (Guinea pig ileum) and in vitro (rabbit jejunum) at different doses ranges from 1 µM to 3 µM.

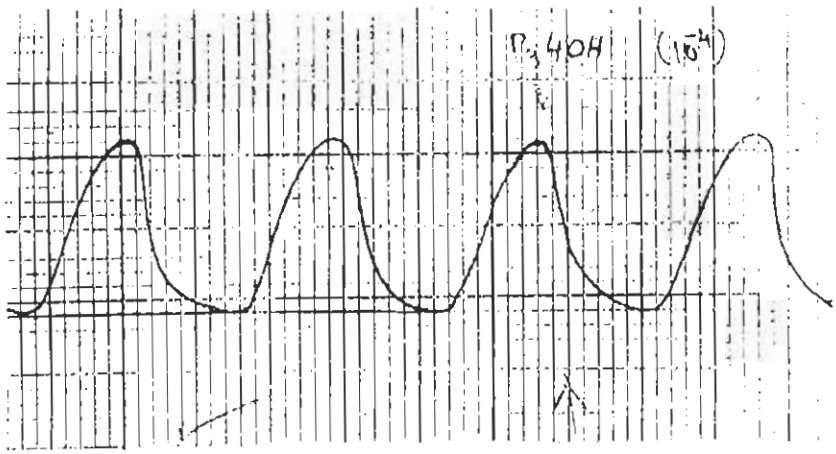
No compound either have any contractile and relaxant activity on intestinal smooth muscles preparations up to very high dose i.e. 3 µM.

EFFECT ON RABBIT INTESTINAL MUSCLE

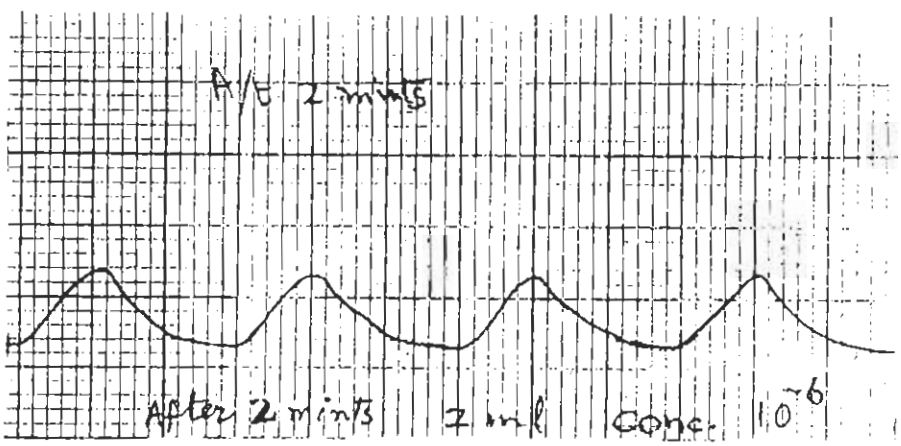
The compound P34OH tested on rabbit intestinal muscle showed that it not only decrease the rate of intestinal rhythmically but also decreases the active tension or force of contraction.

Thus it produces both isotropic and chronotropic effect. The decrease in rate of contraction per minute revealed that the compound slightly acts on beta-receptor and act as beta-agonist which produces inhibitory effect by increasing the duration of response.

This effect was further confirmed by the results of active tension which also decrease after the 2 ml of administration of P34OH at 10 dilution. Thus it is suggested that sarcoplasmic calcium ions also altered by the presence of this compound.



1) Normal Activity of Jejunum



2) Effect of P3 4OH 2 min. after administration of the compound on the activity of jejunum

REFERENCES

Ahmed, F.R., Lawa, G.F., Madani, A.E. and Casy, A.F., *J. Med. Chem.* (1985), **28**, 1947.

Anderson, R.J., Frearson, P. M. and Stern, E. S., *J. Chem. Soc.* (1956), 4088.

Archibald, J.L. and Benke, G.A., *J. Med. Chem. (1974)*, **17**, 736.

Archibald, J.l . Fairbrother, P. and Jackson, J.L., *J. Med. Chem.* (1974), **17**, 739.

Archibald, J.L.. Alps, B.J., Cavalla, J.F. and Jackson, J.L., *J. Med. C'hem.* (1971), **14**, 1054

Archibald, J.L., Baum, T. and Childress, S.J., *J. Med. Chem.* (1970), **13**, 138.

Archibald, J.I ., *J. Heterocycl. Chem.* (1966), **3**, 409.

Avison, W.D. and Morrison, A.l., *J. Chem.* Soc. (1950), 1460.

Bachrach, E.H., Godholm, A.N. and Betcher, A.M.M., *Surgery* (1955), **37**, 440.

Bagley, J.R., Thomas, S.A., Ruclo, F.G., Spencer, H.K., Doorley, B.M., Ossipove, M.H. Jerus.si, T.P., Benvenga, M.J. and Spauciling, T., *J. Med. Chem.* (1991), **34**, 827.

Bagley, J.R., Wynn, F.G., Rudo, F.G., Doorley, B.M. and Spen, H.K., *J. Med. Chem*, (1989), **32**, 663.

Balon Ph.D. Thesis, University of London (1959).

Beckett, A.H. and Casy, A.F., *Bull. Narcot.* (1957), **9**, 37.

Beckett, A.H. and Casy, A.F., *Progr. Med. Chem.* (1965), **4**, 171.

Beckett, A.H. and Greenhill, J.V., *J. Med. Pharm. Chem.* (1961), **4**, 423.

Beckett, A.H., Casy, A.F. anti Kirk, G., J. Med. *Phanm.* (1959). **1**, 37,

Bergel, F. , Hindley, N.C. Morrison, A. L. and Rinderknecht, H., *J. Chem. Soc.* (1944), 269.

Berger, J.G., Davidson, F. and Langford, G.E. *J. Med. Chem.* (1977), **20**, 800.

Blanchi, C. and David, A. *J. Pharm. London* (1960), **12**, 449.

Blair, A.M.J.N. and Stephenson, R.P., Brit. *J. Pharmacol.* (1960), **15**, 247.

Boggiano, G., Petrow, V., Stephenson, O. and Wild, A.M., *J. Chem. Soc.* (1959), 1143.

Bolton, T.B., *Physio/. Rev.* (1979), **59**, 606.

Brading, A.F., *TIPS*, (1991), **2**, 261.

Braenden, O.J., Eddy, N.B. and Halbach, H., *Bull. World. Hlth. Org.* (1955), **13**, 937.

Brine, G.A., Boldt, K.G., Huang, P.T., Sawyer, D.K. and Carroll. F.I. .J. *Heterocycl. Chem.* (1989), **26**, 677.

Buchi, J., Prost, M., Eichenberger, E. and Lieberherr, R., *Helv. Chim. Acta* (1962), **35**, 1527.

Buchi, J., Prost, M., Eichenberger, E. and Lieberherr, R., Helv. Chim. *Acta* (1953), **36**, 819.

Carabateas, P.M., Wetterau, W.F. and Brumbach, L., *J. Med. Chem*, (1963), **6**, 355.

Carlsson, A. *Adv. Biochem. Psychopharmacol.* (1977), **16**, 439.

Caroon, J.M., Clark, R.D. ICluge, A.F. and Whitting, R.L. *J. Med. Chem,* (1981), **24**, 1320.

Casy, A.F. and Armstrong, N.A. *J. Med. Chem,* (1965), **8**, 57.

Casy, A.F. and Huskstep, M.R., *J. Pharm. Pharmacol.* (1988a), **40**, 605.

Casy, A.F. Beckett, A.H., Hall. G.H. and Vallance, D.J. *J. Med. Pharm. Chem.* (1961), **4**, 535.

Casy, A.F. Hassan, M.M.A., Simmonds, A.B. and Stainforth, D. *J. Pharm. Pharmacol.* (1969), **21**, 434.

Casy, A.F., *Med. Res. Rev.* (1982), **2**, 167.

Casy, A.F., Simmonds, A.B. and Staniforth, D. *J. Pharm. Pharmacol.* (1968), **20**, 768.

Chakrabarti, J.L. Foulis, M.J.J., Hotten, T.M. Szinan, S.A. and Told, A. *J. Med. Chem,* (1974), **17**, 602.

Cheng, C., Hanssen, E.B. and Watersn, J.A., *J. Med. Chem,* (1982), **25**, 145.

Clark, R.D. Caroon, J.M., Kluge, A.F. and Okada, M.D. *J. Med. Chem,* (1983), **26**, 657.

Colapret, J.A., Diamantidis, G., Spencer, H.K. Spaulding, T. and Rudo, F.G. *J. Med. Chem,* (1989), **32**, 968.

Davis, M.A., Herr. F., Thomas, R.A. and Charest, M. *J. Med. Chem,* (1967), **10**, 627.

Distasi, L.C. Costa, M. and Mendacolli, S.L.J. *J. Ethanopharmacol.* (1988), **24**, 205.

Eiseib, O. and Schaumann, O. *Deut. Med. Wochschr.* (1939), **65**, 967.

Eipern, B., Carabateas, P., Soria, A. E. and Grumbach, L., *J. Org. Chem.* (1960), **24**, 2045.

Eipern, B., Carabateas, P., Soria, A.E., Gardner, L.N. and Grumbach, L. *J. Amer. Chem. Soc.* (1959), **81**, 3784.

Eipern, B., Gardner, L.N. and Grambuch, L. *J. Amer. Chem. Soc.* (1957), **79**, 1951.

Eipern, B., Wetteran, W., Carbateas, Ph. and Grumbach, L. *J. Amer. Chem. Soc.* (1958), **80**, 4916.

Fancher, D.E. Hayao, S., Strychker, W.G. and Sancillio, L.F. *J. Med. Chem.* (1964), **7**, 721.

Feldman, P.L., James, M.K., Brackeen, M.F., Lutz, M.W. and Leighton, H.J. *J. Med. Chem.* (1991), **34**, 2202.

Finch, L. *Eur. J. Pharmacol.* (1975), **33**, 409.

Fearson, P.M. and Stern, E.S. *J. Chem. Soc.* (1958), 3062, 3065.

Frearson, P.M., Hardy, D.G. and Stern, E.S. *J. Chem. Soc.* (1960), 2103.

Gerzon, K. and Kall, D. *J. Med. Chem.* (1967), **10**, 189.

Gerzon, K., Donald, J., Tobias, Sr., Holmes, R.E., Rathbun, R.E. and Kattau, R.W. *J. Med. Chem.* (1967), **10**, 6033.

Gerzon, K., Eriks, V., Krumkains, E.V., Richard, L., Brindle, R.L., Fredrick, J., Marshall, F.J. and Marya, A. *J. Med. Chem.* (1963), **6**, 760.

Gilani, A.H. and Cobbin, L.B. *J. Pharmacol.* (1986), **332**, 16.

Gilani, A.H., Janbaz, K.H., Lateef, A. and Zaman, M. *Phytother. Res.* (1994), **8**, 161.

Gilani, A.H., Khalid, H. and Anisa L. *Phytother. Res.* (1994), **8**, 161.

Glazebrook, A.J. and Branwood, A.W. *Lancet* (1945), **249**, 528.

Green, A.F. and Ward, N.Z. *Brit. J. Pharmacol.* (1956), **11**, 32.

Hacksell, U., Arvidssor, L.E. Svensson, U., Nilsson, J.L.G. Sanchez, D., Wikstrom, H., Lindberg, P., Ijorth, S. and Carlsson, A., *J. Med. Chem.* (1981), **24**, 1475.

Haleem, D.J., Kennett, G.A. and Curzon, G., *J. Neural. Trans.* (1990), **79**, 93.

Henkel, J.G.H., Hane, J.T. and Gianutsos, G., *J. Med. Chem.* (1982), **25**, 51.

Huang, B.S., Terrell, R.C., Deutsche, K.H., Kudzma, L.V. and Lalinde, N.L. **U.S. Patent** (1986), 584304.

Hutchinson, A.J., de Hesus, R., Williams, M. , Simke, J. L., Neals, R.F., Jackson, R.H., Ambrose, F., Barbaz, B.J. and Sills, M.A., *J. Med. Chem.* (1989), **32**, 2221.

Iorio, M.A., Casy, A.F. and May, E.L., *Eur. J. Med. Chem.* (1975), **10**, 178.

Iorio, M.A., Reymer, T.P. and Frigeni, V., *J. Med. Chem.* (1987), **30**,1906.

Iorio, M.A. and Klee, W.A., *J. Med. Chem.* (1977), **20**, 309.

Ishikawa, K. and Megaugh, J.L., *J. Chromatogr. Biomed. Appl.* (1982), **229**, 35.

Jacobson, A.E., May, E.L. and Sargent, L.J., *Medicinal Chemistry*, Burger A., Ed., Wiley Interscience, New York (1970), Part **II**, p. 1327.

Jacoby, R.L., Nieforth, K.A. and Willette, R.E. *J. Med. Chem.* (1974), **17**, 453.

Janssen Pharmaceuticals, **U.S. Patents.** (1967), 3318900 (1975), 3894030.

Janssen, P.A.J. and Eddy, N.B., J. *Med. Pharm. Chem.* (1960a), **2**, 31.

Janssen, P.A.J., **U.S. Patent.** (1964), 3141823.

Janssen, P.A.J., *Brit. J. An'aesth.* (1962a), **34**, 260.

Janssen, P. A.J., *An'aesthesist* (1962b), **11**, 1.

Janssen, P.A.J., *Chem. Abstr.* (1964), **61**, 10689.

Janssen, P.A.J., *Chem. Abstr.* (1965), **63**, 9952.

Janssen, P.A.J., *J. Pharm. Pharmacol.* (1961), **13**, 513.

Janssen, P.A.J., Jageneau, A.H.M., Demoen, P.J.A., van der Westeringh, C., Raeymaekers, A.H.M., Wouters, M.S.J., Sanczuk, S., Hermans, B. and Loomans, J.L.M., *J. Med. Pharn. Chem.* (1959a), **1**, 105.

Janssen, P.A.J., Jageneau, A.H.M. and Huygens, J., *J. Med. Pharm. Chem.* (1959b), **1**, 299.

Janssen, P.A.J. , Jageneau, A.H.M., Demoen, P.J.A., van de Westeringh, C., de Canniere, J.H.M., Raeymaekers, A.H.M., Wouters, M.S.J., Sanczuk, S. and Hermans, B.K.F., *J. Med. Pharm. Chem.* (1959c), **1**, 309.

Janssen, P.A.J., Jageneau, A.H., Van Proosdij-Hartzema, E.G. and de Jongh D.K., *Acta Physiol. Pharm. Neerl.* (1958), **7**, 373.

Janssen, P.A.J. Jageneau, A.H.M., Demoen, P.J.A., van de Westeringh, C., de Canniere, J.H.M., Raeymaekers, A.H.M., Wouters, M.S.J., Sanczuk, S. and Hermans, B.K.F., J. *Med. Pharm. Chem.* (1960b), **2**, 271.

Janssen, P.A.J., **U.S. Patent** (1962c), 3161644.

Janssen, P.A.J., Van der Westeringh, C., Jagenean, A.H.M., Demooen, P.J.A. and Hermans, B.K.F., *J. Med. Pharm. Chem.* (1959d), **1**, 281.

Janssen, K.A., Lundquist, E., Rekling, E. and Wolfbrandt, C.G., *Dansk Tiddsskr Farm.* (1943), **17**, 173.

Jilek, J.O., Seidlova, V., Svetek, E. and Protiva, M., *Monatsh. Chem.* (1965), **96**, 182.

Kagi, H. and Miescher, K., *Helv. Chem. Acta* (1949), **32**, 2489.

Klein, W., Bach, W. and Mutschler, E., *Arch. Pharm.* (1975), **308**, 910.

Klioze, S.S. and Novick, W.J., *J. Med. Chem.* (1978), **21**, 400.

Kohut, A. and Nicak, A., *Activ'e Nerv. Super.* (1974), **16**, 130.

Krapcho, J. and Turk, C.F., *J. Med. Chem.* (1963), **6**, 729.

Kudzma, L.V., Severnak, S.A., Benvenga, M.J., Ezell, E.F. Ossipov, M.H. Rudo, F.G. Spencer, H.K. and Spaudling, T.C., *J. Med. Chem.* (1989), **32**, 2534.

Kugita, H., Inoue, H., Oine, T. and Hayashi, G., *J. Med. Chem.* (1965), **8**, 313.

Kugita, H., Inoue, H., Oine, T. Hayashi, G. and Nurinoto, S., *J. Med. Chem.* (1963), **7**, 298.

Lalinde, N., Moliterni, J., Wright, D. and Spencer, H.K., *J. Med. Chem.* (1990), **33**, 2876.

Landa, S., Machacek, V. and Mzourek, J., *Chem. Listy.* (1933), **27**, 415, 443.

Lasagna,L. and Beecher, HJ.K., *J. Pharmacol. Expt. Ther.* (1954), 30.

Lawson, J.A., Cheng, A., DeGraw, J., Frenkling, G., Uyeno, E., Toll, L. and Loew, G.H., *J. Med. Chem.* (1988), **31**, 2015.

Lednicer, D. and Mitscher, L.A., *Organic Chemistry of Drug Synthesis*, John Wiley and Sons, New York (1977 and 1980), Vol. **1** and **2**, pp. 298 and 328.

Loew, G.H., Lawson, J.A., Uyeno, E.T., Tool, L., Frenking, G., Polgar, W., Ma, L.Y.Y., Camerman, N. and Camerman, A., *Mol. Pharmacol.* (1988), **34**, 363.

Loullis, C.C., Hingtgen, J.N., Shea, P.A. and Carlson, M.H., *Pharmacol. Biochem. Behav.* (1980), **12**, 959.

Macdonald, A.D., Woolfe, G., Bergel, F., Morrison, A.L. and Rinderknecht, H.J., *Brit. J. Pharmacol.* (1956), 1.

Mailey, E.A. and Day, A.R., *J. Org Chem.* (1957), **22**, 1061.

Maillard, J., Delaunary, P., Langlois, M. and Vo Van, T., *Bull. Soc. Chim. Fr.* (1970), 1398.

Maillard, J., Langlois, M., Delaunary, P.O., Vo Van, T., Chenu, J., Morin, R., Bendarkate, M., Manuel, C. and Motosso, F., *J. Med. Chem.* (1972), **15**, 1123.

Maillard, J., Langlois, M., Vo Van, T., Delaunary, P., Morin, R., Benharkate, M., Manuel, C. and Motosso, F., *Eup. J. Med. Chem.* (1973), **8**, 393.

Maillard, J., Langnois, M., Delaunay, T., Vo Van, T., Gareta, G., Morin, R., Eskenazi, P., Benharkate, M., Manuel, C. and Motosso, F., *Eur. J. Med. Chem.* (1972), **7**, 458.

Maillard, J., Langnois, M., Vo Van, T., Morin, R., Benharkate, M. and Plc, P. *Eur. J. med. Chem.* (1974), **9**, 128.

Mashkovskii, M.D. and Abramova, P.N., *Pharm. and Toxic.* (1956), **19**. 26.

Mauleon, D., Pujol, M.D. and Rosell, G., *J. Med. Chem.*, (1988), **31**, 2122.

McElvain, S.M. and Barnett, M.D., J. *Amer. Chem. Soc.*, (1956), **78**, 3140.

Mcleod, L.C., *Pharmacological Experiments on Intact Preparations*, E. & S. Livingstone, Edinburg (1970), p. 63.

McMillan, F.H., Kin, K.A., McMillain, C.B. and King, J.A., *J. Amer. Chem. Soc.* (1956), **78**, 4077.

Marlevede, E. and Levis, S., *Arch. Int. Pharmacodyn.*, (1958), **115**, 213.

Millar, R.A. and Stephenson, R.P., *Brit. J. Pharmacol.* (1956), **11**, 27.

Minor, W.F., Hoekstra, J.B., Fisher, D. and Sam, J., *J. Med Chem.* (1962), **5**, 96. *Ind. Chim. Belge.*

Morren, H. and Strubbe, H., *Ind. Chim. Belge.* (1957), **22**, 795.

Nazarov, I.N., Mashkoviskii, M.D., Rudenko, V.A. and Prostakov, E., *Klin. Med. Mosk.* (1952), **30**, 60.

Nazarov, I.N., Raigorodskaya, V. Ya and Rudenko, V.A., *Bull. Acad. Sci.* (1949), 504.

Newberne, J.W., Gibson, J.P. and Newberne, P.M., *Toxicol. Appl. Pharmacol.* (1967), **10**, 233.

Norton, S. and Phillips, A.P., *Nature.* London (1953), **172**, 867.

Obase, H., Nakamizo, N., Takai, H., Teranishi, M., Kubo, K., Shuto, K., Kasuya, Y. and Shigenobu, K., *Chem. Pharm. Bull.* (1982), **30**, 474.

Okeeffee, R. and Sharman, D.F., *Br. J. Pharmacol.* (1970), **38**, 287.

Ong. H.H. and Profitt, J.A.,.*J. Med. Chem.* (1979), **22**, 834.

Orahovats, P.D., Lehman, E.G. and Chapin, E.W., J. *Pharmacol.* (1957), **119**, 26.

Perrine, T.D. and Eddy, N.B., *J. Org. Chem.* (1956), **21**, 125.

Phillips, A.P., *J. Amer. Chem. Soc.* (1954), **76**, 2211.

Phillips, A.P., *J. Amer. Chem. Soc.* (1957), **79**, 2836.

Phillips, A.P., *J. Amer. Chem. Soc.* (1957), **79**, 5754.

Rall, D.P. and Zubrod, C.G., *Am. Rev. Pharma.* (1962), **2**, 115.

Randall, L.A. and Lehman, G.J., *J. Pharmacol.* (1948), **93**, 314.

Rapala, R.T., Kraay, R.J. and Gerzon, K., *J. Med. Chem.* (1965), **8**, 580.

Riley, T.N., Hale, D.B. and Wilson, M.C. *J. Pharm.* Sci. (1973), **62**, 983.

Rogers, M.E., Wilkison, D.S., Thweatt, J.R. and Halenda, S.P., *J. Med. Chem.* (1980), **23**, 688.

Roberts, M.H.T., *Drug Design Del.* (1989), **4**, 77.

Ross, J.B., Nora, S.K., Dennis, F. and Neumeyer, J.L., *Life Science* (1986), **39**, 1765.

Roth, R.H., *Commun. Psychopharmacol.* (1979), **3**, 429.

Royds, R.B., Coltart, D.C. and Lockhart, J.D.F., *Clin. Pharmacol. Ther.* (1972), **13**, 380.

Saify, Z.S., Ph.D. Thesis, University of London (1971).

Salva, P.S., Hite, G.J., Heyman, R.A. and Gianutsos, G.J., *J. Med. Chem.* (1986), **29**, 2111.

Samanin, R., Miranda, F. and Mennini, T., In *Proceedings of Ist RIC Farmacol. Marip. Negri.* (1978), p. 523, Milan, Italy.

Sawyork, J., *Can. J. Physiol. Pharmacol.* (1989), **67**, 975.

Schaechter, J.D. and Wurtman, R.J., *Brain Res.* (1990), **532**, 203.

Scriabine, A.P. *"Pharmacology of Antihypertensive Drugs"*, (Ed. by A. Seriabine, Raven Press), New York, (1980), p. 43.

Shapiro, S.L., Solowy, H. and Freedman, L., *J. Amer. Chem. Soc.* (1958), **80**, 2743.

Shapiro, S.L. Solowy, H., Shapiro H. and Freedman, L., *J. Org Chem.* (1960), **25**, 291.

Skirboll, L.R. Grace, A.A. and Bunney, B.S., *Science* (1979), **206**, 80.

Spielman, M.A. (1950), **U.S. Patent,** 2500714.

Sterling Drug Inc. (1959), **U.S. Patent,** 2901487.

Strombom, U., *Acta Physiol. Scand.*, Suppl. (1975), 431.

Takai, H., Obase, H., Nakamizo, N., Teranishi, M., Kubo, K., Shuto, K. and Hashimoto, T., *Chem. Pharm. Bull.* (1985a), **33**, 1104.

Takai, H., Obase, H., Nakamizo, N., Teranishi, M., Kubo, K., Shuto, K., Kassuya, Y., Shigenobu, K., Hashikami, M. and Karashima, N., *Chem. Pharm. Bull.* (1985b), **33**, 1116.

Takai, H., Obase, H., Teranishi, M., Karasawa, A., Kubo, K., Shuto, K., Kasuya, Y., Hashikami, M., Karashima, N. and Shigenobu, K., *Chem. Pharm. Bull.* (1985c), **33**, 1129.

Tollenaere, J.P. and Janssen, P.A.J., *Med. Res. Rev.* (1988), **8**, 1.

Turner, R.A. and Hebborn, P., *Screening Methods in Pharmacology*, **1**, Aademic Press, New York (1971), p. 114.

Van Daele, P.G.H., Dr. Bruyn, M.F.L. Boey, J.M., Sanczuk, S., Agren, J.T.M. and Janssen, P.A.J., *J. Arzneim. Forsch. Drug Res.* (1976), 26, 1521.

Van Zweiten, P.A., *Arch. Int. Pharmacodyn. Ther.* (1975), **215**, 104.

Voldeng, A.N., Bradley, C.A., Kee, R.D., King, E.L. and Medler, F.L., *J. Pharm. Sci.* (1968), **57**, 1053.

Weijlard, J., Orahovats, P.D., Sullivan, A.P. Jr., Purdue, G., Heath, F.K. and Pfister, K., *J. Amer. Chem. Soc.* (1956), 78, 2342.

Weiner, N., Goodman and Gilman's *Pharmacological Basis of Therapeutics*, ed. by A.G. Gilman, L.S. Goodman, T.W. Rall and F. Murad, MacMillan, New York (1985), pp. 145.

Wellens, D., Snoeck, L., De Reese, R., Kruger, R., Van De Water, A., Wouters, L. and Reneman, R. S., *Arch. Int. Pharmacodyn. Ther.* (1975), **215**, 119.

Wellens, D., De Wilde, A., Van Bogaert, A., Van Bogaert, P.P., Wouters, L., Reneman, R.S. and Janssen, P.A.J., *Arch. Int. Pharmacodyn. Ther.* (1975), **215**, 91.

Wellens, D., Van Nueten, J.M. and Janssen, P.A.J., *Arch. Int. Pharmacodyn. Ther.* (1975), **213**, 334.

Wikstrom, H., Sanchez, D., Lindberg, P., Hacksell, U., Arvidsson, L.E., Johansson, A.M., Thorberg, S.O., Nilsson, J.L.G., Sevensson,

K., Hjorth, S., Clark, D. and Carlsson, A., *J. Med. Chem.* (1984), **27**, 1030.

Witiak, D.T., Vishnuvajjala, B.R., Gupta, T.K. and Gerald, M.C., *J. Med. Chem.* (1976), **19**, 40.

Zimmerman, D.M., Cantrell, B.E., Swartzendruber, J.K., Jones, N.D., Mendelson, L.G. and Nickander, J.D., *J. Med. Chem.* (1988), **31**, 555.

APPENDIX

LIST OF FIGURES

7	7	The effects of N-methylphenacyl derivatives of piperidine (I, 3 , 5 , 6 and 8) on mice brain 5HT levels.
8	8	The effects of N-methylphenacyl derivatives of piperidine (2 4 , 7 , and 9) on mice brain 5HT levels.
9	9	The effects of N-methylphenacyl derivatives of piperidine (I, 3 5 6 and 8) on mice brain 5HIAA levels.
10	10	The effects of N-methylphenacyl derivatives of piperidine (2 4 , 7 , and 9) on mice brain 5HIAA levels.
11	11	Analgesic effect of compound (1) in mouse tail immersion method.
12	12	Analgesic effect of compound (2 in mouse tail immersion method.
13	13	Analgesic effect of compound (3) in mouse tail immersion method.
14	14	Analgesic effect of compound (4) in mouse tail immersion method.

15	15	Analgesic effect of compound (5) in mouse tail immersion method.
16	16	Analgesic effect of compound (6) in mouse tail immersion method.
17	17	Analgesic effect of compound (7) in mouse tail immersion method.
18	18	Analgesic effect of compound (8) in mouse tail immersion method.
19	19	Analgesic effect of compound (9) in mouse tail immersion method.

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8	8	Analgesic effect of Compound '6' in mouse tail immersion method.
9	9	Analgesic effect of Compound '7' in mouse tail immersion method.
10	10	Analgesic effect of Compound '8' in mouse tail immersion method.
11	11	Analgesic effect of Compound '9' in mouse tail immersion method.
12	12	Brine shrimp assay results for (I) and its phenacyl derivatives (2-9)

LIST OF SUBSTANCES

S. No.	Name of Compound
1.	1-Methyl-1 (4-methoxy) phenacyl-4-hydroxy piperidinium bromide (I)
2.	1-Methyl-1 (4'-bromo) phenacyl-4-hydroxy piperidinium bromide (2)
3.	1-Methyl-1 (3,4-dihydroxy) phenacyl-4-hydroxy piperidinium chloride (3)
4.	1-Methyl-1 (3-methoxy) phenacyl-4-hydroxy piperidinium bromide (4)
5.	1-Methyl-1 (4-nitro) phenacyl-4-hydroxy piperidinium bromide (5)
6.	1-Methyl-1 (2,4-difluoro) phenacyl-4-hydroxy piperidinium chloride (6)
7.	1-Methyl-1 (4-chloro) phenacyl-4-hydroxy piperidinium bromide (7)
8.	1-Methyl-1 (4-fluoro) phenacyl-4-hydroxy piperidinium bromide (8)
9.	1-Methyl-1 (4-methyl) phenacyl-4-hydroxy piperidinium bromide (9)