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# Synthesis and Pharmacological Evaluation of New Ethyl Esters of N-Acyl Amino Acids as CNS Agents

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The ethyl esters **1a–1j** and **2a–2j** of *N*-acyl amino acids were synthesized by the DCC method. The compounds were screened for their monoamine oxidase (MAO) inhibitory activity (*in vitro*) and various CNS activities (*in vivo*). Some compounds showed promising MAO inhibitory and anti-depressant activities. The compounds did not produce acute neurological deficits and have low toxicity.

**Synthese und pharmakologische Prüfung neuer N-acylierter Aminosäureester als ZNS-wirksame Verbindungen**

Die N-acylierten Aminosäureester **1a–1j** und **2a–2j** wurden nach der DCC-Methode synthetisiert. Die erhaltenen Verbindungen wurden in vitro auf ihre MAO-hemmende Aktivität und in vivo auf ZNS-Aktivität getestet. Einige der geprüften Verbindungen besitzen interessante pharmakologische Eigenschaften.

Amino acids are precursors for the biosynthesis of various neurotransmitters localized in CNS and peripheral tissues e.g. tryptophan for serotonin, phenylalanine for adrenaline etc.[1]. These neurotransmitters play an important role in the functioning of CNS and their deficiencies have been implicated in certain behavioural disorders like depression[2]. There are several reports in the literature stating that amino acid moieties have been successfully linked to a biologically active nucleus and the resulting compounds have shown increased biological activity. Several derivatives of quinoline[3] and piperidine[4] are reported to possess CNS activity. Therefore, it was thought worthwhile to link amino acids to substituted quinoline and piperidine and to evaluate the products for their CNS activities. All the compounds were assayed for their in vitro MAO inhibitory activity and various CNS activities in vivo.

For the synthesis of **1a–1j** and **2a–2j** the starting material amino acid ethyl ester hydrochlorides and peptide ethyl ester hydrochlorides were prepared by the esterification of the corresponding amino acids and peptides. Condensation of quinoline-2-carboxylic acid or piperidine-4-carboxylic acid with the appropriate amino acid ester hydrochloride and peptide ester hydrochloride in (dry) methylene chloride, triethylamine and dicyclohexyl carbodiimide gave the desired compounds **1** and **2**.

** Part of this will be incorporated in the Ph.D. thesis of *G. Sathi*.

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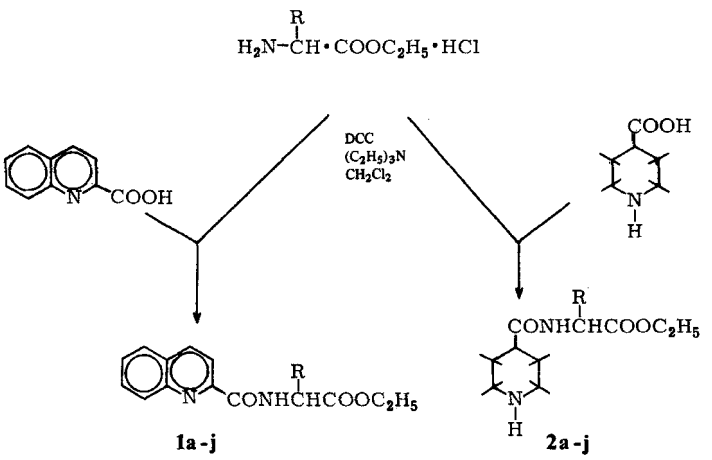


## Results and Discussions

### Biochemical Studies

All the 20 compounds were assayed for their *in vitro* MAO inhibitory activity as reported in table 1 and 2. All compounds showed MAO inhibition of varying degree at $1 \cdot 10^{-4}$ *M* final concentration. Out of these, 5 compounds **(1b, 1g, 1h, 1j** and **2h)** showed more than 70 % inhibition at this concentration.

An examination of enzyme inhibitory activity in relation to chemical structure, showed that the compounds with amino acid substituents viz. phenyl alanine, α-alanine, valine showed increased MAO inhibitory activity. The most active compound which showed 85.9 % MAO inhibition is **1h** with a phenyl alanine substituent at position 2 of the quinoline nucleus. The quinoline compounds were found to be more active than the piperidine compounds.

### Pharmacological Studies

Pharmacological activities of the compounds were compared with their starting materials viz. piperidine-4-carboxylic acid (P) and quinoline-2-carboxylic acid (Q) (table 3).

All the test compounds increased the spontaneous locomotor activity. The maximum (26.3 %) increase was observed with compound **1h** and the minimum (2.1 %) with compound **1j**. Awareness was increased by compounds **1g** and **1h**, with the rest of the compounds awareness remained unchanged. None of the compounds showed any other behavioural effect.

Reserpine induced a decrease of 80.9 % in locomotor activity from control, while in animals pretreated with test compounds the decrease was smaller. The maximum antagonism of reserpine hypokinesia was observed with compound **1h** (only 56.4 % decrease in locomotor activity). Ptosis was reduced maximally (4.8 scores) by compound **1h**. Compound **1g** and **2h** reversed the ptosis to the score of 6.2 and 6.4 resp. Diarrhoea was

inhibited by compound **1g, 1h** and **2h** whereas compounds **1b** and **1j** did not effect ptosis and diarrhoea.

In L-dopa pretreated mice locomotor activity was enhanced by all the compounds. The maximum increase (41.1 %) was observed with compound **1h**, while with other compounds the increase ranged from 15.2 % and 33.0 %. Compound **1h** caused a 3 times increase in piloerection. Two fold increase in piloerection was observed with compounds **1g** and **2h**. Compounds **1g, 1h** and **2h** showed two times more stereotype behaviour than control.

Compounds **1b** and **1j** did not effect piloerection and stereotype. Fighting was observed in compound **1g**.

None of the compounds showed any acute neurological deficits upto the 1000 mg/kg i.p.

It is clearly evident from the results that substitution of α-alanine **(1g)** and phenyl alanine **(1h)** at position C-2 in the quinoline nucleus and phenyl alanine **(2h)** at position C-4 of the piperidine nucleus causes a marked anti-depressant activity while substitutions of glycyl-L-leucine **(1j)** and valine **(1b)** at C-2 position in the quinoline nucleus do not influence activity. The maximum antidepressant activity was observed with compound **1h**. The compounds **1h, 1g** and **2h** showed better activity than the parent quinoline or piperidine. This indicates that the substitution of amino acids or peptides in these compounds causes an increase in the activity. The antidepressant activities of these compounds correlate well with their in vitro MAO inhibition. Furthermore, these compounds are quite safe because they did not produce any acute neurological deficits and their $ALD_{50}$ values were higher.

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## Experimental

*MP*: in open capillaries (uncorr.). *IR-spectra*: Perkin-Elmer 177 spectrophotometer in KBr.

Amino acid ethyl ester hydrochlorides[5] and peptide ethyl ester hydrochlorides[6] were prepared by the reported methods.

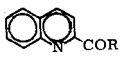
### *Synthesis of Compounds* **1a–1j** *and* **2a–2j**

1.01 g, (0.01 mole) triethylamine was added to a cooled solution of the appropriate amino acid ethyl ester hydrochloride/peptide ethyl ester hydrochloride (0.01 mole) in methylene chloride (dry, 25 ml).

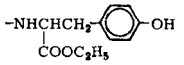
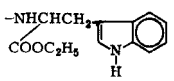
1.74 g, (0.01 mole) quinoline-2-carboxylic acid or 1.2 g (0.01 mole) piperidine-4-carboxylic acid and 2 g, (0.01 mole) N,N-dicyclohexyl carbodiimide were added to the above reaction mixture and stirred for 10 h at room temp. The separated dicyclohexyl urea was filtered off and the filtrate was washed with 1N-HCl, 1N-$Na_2CO_3$ and finally with a saturated solution of sodium chloride; then dried over sodium sulphate and evaporated.

The residue obtained was dissolved in hot benzene and left for 2 h, then the benzene was removed, the residue washed with petroleum ether and the solid obtained recrystallized from an appropriate solvent. The compounds thus prepared are reported in Tables 1 and 2.

**Table 1:** *Compounds* **1a–1j**



**1a-1j**

| Sl. No. | R | Molecular formula (A)* | M.P. °C | Yield % | Recrystallising solvent (B) | % MAO inhibition at $1 \cdot 10^{-4}$ M (C) |
|---|---|---|---|---|---|---|
| 1a | $-NHCH_2COOC_2H_5$ | $C_{14}H_{14}N_2O_3$ | 120 | 50 | B/C | 64.9 |
| 1b | $-NHCH(COOC_2H_5)-CH(CH_3)_2$ | $C_{17}H_{20}N_2O_3$ | 185 | 45 | A | 76.6 |
| 1c | $(CH_3)_2CHCH_2-CH(NH)COOC_2H_5$ | $C_{18}H_{22}N_2O_3$ | 111 | 50 | A | 67.4 |
| 1d |  **1d** | $C_{21}H_{20}N_2O_4$ | 95 | 55 | B/C | 67.9 |
| 1e |  **1e** | $C_{23}H_{31}N_3O_3$ | 115 | 60 | B/C | 66.2 |
| 1f | $-NHCH_2CH_2COOC_2H_5$ | $C_{15}H_{16}N_2O_3$ | 165 | 60 | B/C | 67.8 |
| 1g | $-NH-CH(CH_3)-COOC_2H_5$ | $C_{15}H_{16}N_2O_3$ | 215 | 60 | A | 72.3 |
| 1h | $C_6H_5CH_2CH(NH)-COOC_2H_5$ | $C_{21}H_{20}N_2O_3$ | 192 | 55 | B/C | 85.9 |
| 1i | $-NHCH_2CONHCH_2COOC_2H_5$ | $C_{16}H_{17}N_3O_4$ | 110 | 50 | B/C | 57.2 |
| 1j | $-NH-CH_2CONH-CH(CH_2-CH(CH_3)_2)-COOC_2H_5$ | $C_{20}H_{25}N_3O_4$ | 134 | 55 | A | 71.4 |

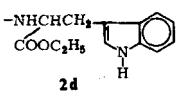
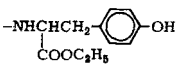
* (A) All the compounds gave satisfactory elemental analysis. **1a** and **1i**: I.R. (KBr) $\upsilon$ max C=O (1680 $cm^{-1}$), C=O of ester linkage 1710 $cm^{-1}$), NH (3200 $cm^{-1}$) and C=N (1600 $cm^{-1}$).

(B) A – alcohol, B – benzene, C – petroleum ether.

(C) Compounds were dissolved in propylene glycol and tested at final concentration of $1 \cdot 10^{-4}$ *M*, each experiment was done in duplicate, values in the table are mean of the two separate experiments.

**Table 2:** *Compounds* **2a–2j**

**2a-2j**

| Sl. No. | R | Molecular formula (A)* | M.P. °C | Yield % | Recrystallising solvent (B) | % MAO inhibition at $1 \cdot 10^{-4}$ M (C) |
|---|---|---|---|---|---|---|
| **2a** | $-NHCH_2COOC_2H_5$ | $C_{10}H_{18}N_2O_3$ | 121 | 30 | A | 63.6 |
| **2b** | $-(NH)-CH(COOC_2H_5)CH(CH_3)_2$ | $C_{13}H_{24}N_2O_3$ | 136 | 35 | B/C | 53.7 |
| **2c** | $(CH_3)_2CHCH_2CH(NH)-COOC_2H_5$ | $C_{14}H_{26}N_2O_3$ | 142 | 30 | B/C | 64.4 |
| **2d** |  **2d** | $C_{19}H_{25}N_3O_3$ | 190 | 30 | A | 68.7 |
| **2e** |  **2e** | $C_{17}H_{24}N_2O_4$ | 172 | 40 | A | 63.6 |
| **2f** | $-NHCH_3CH_2COOC_2H_5$ | $C_{11}H_{20}N_2O_3$ | 105 | 45 | B/C | 54.6 |
| **2g** | $-NHCH(CH_3)COOC_2H_5$ | $C_{11}H_{20}N_2O_3$ | 192 | 40 | B/C | 56.3 |
| **2h** | $C_6H_5CH_2CH(-NH)-COOC_2H_5$ | $C_{17}H_{24}N_2O_3$ | 163 | 40 | B/C | 71.8 |
| **2i** | $-NHCH_2CONHCH_2COOC_2H_5$ | $C_{12}H_{21}N_3O_4$ | 121 | 45 | B/C | 66.1 |
| **2j** | $-NH-CH_2-CONH-CH(CH_2-CH(CH_3)_2)-COOC_2H_5$ | $C_{15}H_{29}N_3O_3$ | 154 | 35 | B/C | 62.5 |

(A)* All compounds gave satisfactory elemental analysis **2a** and **2i**: IR (KBr) ν max C=O (1680 $cm^{-1}$), C=O of ester linkage (1710 $cm^{-1}$) and NH (3200 $cm^{-1}$).

(B) A – alcohol, B – benzene, C – petroleum ether.

(C) The compounds were dissolved in propylene glycol, and tested at the final concentration of $1 \cdot 10^{-4}$ *M*, each experiment was done in duplicate, the values in the table are mean of two separate experiments.

**Table 3:** *Antidepressant Activity*

| Test Compound 100 mg/kg i.p. | General Behaviour | | Antidepressant activity | | | | | | | ALD$_{50}$ mg/kg i.p. |
|---|---|---|---|---|---|---|---|---|---|---|
| | Spontaneous locomotor activity (counts) | Awareness | Reserpine reversal | | | L-dopa potentiation | | | | |
| | | | Locomotor activity (counts) | Ptosis (score) | Diarrhoea | Locomotor activity (counts) | Pilo-erection | Stereo-typy | Fighting | |
| Control | 95 | + | (−)80.9 | 8.0 | +++ | 112 | + | + | − | − |
| **1b** | (+) 3.2 | + | (−)75.5 | 8.0 | +++ | (+) 17.1 | + | + | − | > 1000 |
| **1g** | (+) 21.1 | ++ | (−)69.1 | 6.2 | ++ | (+) 33.01 | ++ | ++ | + | > 1000 |
| **1h** | (+) 26.3 | ++ | (−)56.4 | 4.8 | + | (+) 41.1 | +++ | ++ | − | > 1000 |
| **1j** | (+) 2.1 | + | (−)78.2 | 8.0 | +++ | (+) 15.2 | + | + | − | > 1000 |
| **2h** | (+) 15.8 | + | (−)72.7 | 6.4 | ++ | (+) 23.2 | ++ | ++ | − | > 1000 |
| **P** | (+) 6.8 | + | (−)72.5 | 6.4 | ++ | (+) 14.6 | + | + | − | − |
| **Q** | (+) 1.7 | + | (−)78.7 | 8.0 | +++ | (+) 1.2 | + | + | − | − |

(−) % decrease in locomotor activity from control values

(+) % increase in locomotor activity from control values.
Test compounds were administered as suspension in gum acacia.

## Biochemical Activity

*Determination of monoamine oxidase inhibitory activity*

MAO activity was determined by the spectrophotofluorometric method of *Krazl*[7] using partially purified rat brain preparation (16,000 · g sedimented particles) as an enzyme source and kynuramine as a substrate at a final concentration of $1 \cdot 10^{-4} M$. Optical density was observed at 315 nm and fluorescence at 380 nm in a fluorometer after suitable dilution.

*Pharmacological Activities*

Albino mice of either sex weighing 20–30 g were used in this study, compounds were administered intraperitoneally in a dose of 100 mg/kg i.p.

*Observation of General Behavioural Effect*

Spontaneous locomotor activity (counted by photoactometer) body posture, gait, awareness, abnormal movements, reflexes, autonomic manifestations (lacrimation, salivation, defaecation and urination) and analgesia were observed after 4 h of administration of the compounds.

*Antidepressant Activity*

Antidepressant activity was judged by reversal of reserpine effects (hypokinesia, ptosis and diarrhoea) and potentiation of L-dopa effects (locomotor activity, stereotype, piloerection and fighting) according to *Chessin* et al.[8]. Reserpine (5 mg/kg i.p.) and L-dopa (100 mg/kg i.p.) were administered 3 h after injection of the test compounds.

*Acute Toxicity Test*

Acute neurological deficits were observed according to *Swinyard* et al.[10]. ALD$_{50}$ was determined according to the method of *Smith*[11].

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## Isomere des Benzomorphans, 3. Mitt.[1]

# NMR-Spektroskopische Konfigurationsbestimmung stereomerer Octahydrobenzo[*f*]isochinoline

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Eingegangen am 10. August 1981

Die Konfiguration der stereoselektiv erhaltenen Titelverbindungen **2** wird durch Vergleich mit den Modellverbindungen **5** bewiesen.

**Isomers of Benzomorphane, III[1]: Determination of the Configuration of Octahydrobenzo[*f*]isoquinolines by NMR Spectroscopy**

The configurations of the title compounds, prepared by stereoselective synthesis, were determined by NMR spectroscopy using the model compounds **5** for comparison.

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Im Rahmen unserer Untersuchungen über intramolekulare Aromatenalkylierungen haben wir vor einiger Zeit zeigen können, daß die Cyclisierung des Tetrahydropyridins **1a** stereoselektiv zum *cis*-3,10b-Dimethyl-1,2,3,4,4a,5,6,10b-octahydrobenzo(f)isochinolin **(2a)** führt[2]. Dementsprechend war für einige inzwischen analog synthetisierte Hydroxy-Derivate **2b–2f**[3] die selbe Konfiguration zwar wahrscheinlich, aber bisher noch nicht bewiesen; die vorliegende Arbeit enthält die noch ausstehende stereochemische Zuordnung für die hydroxylierten Titelverbindungen **2**.

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