# Biological Sciences Section

Pakistan J. Sci. Ind. Res., Vol. 16, Nos. 3–4, June–August 1973

## THE BIOCHEMORPHOLOGY OF CYCLOBUTANECARBOXIMIDES

### Part II*

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(Received April 25, 1973)

**Abstract.** Several *N*-acylcyclobutanecarboxamides have been synthesized and examined for general central nervous system depressant properties, barbiturate potentiation, myorelaxant, antitremorine and anticonvulsant potency. Water solubility appears to play a major role in the activity of these compounds. *N*-4-Hydroxybutyrylcyclobutanecarboxamide, the most potent myorelaxant of the series, appears to be more potent than most of the clinically used strychnine antagonists. *N*-Methacrylyl, *N*-phenylacetyl, *N*-3,5-dimethoxybenzoyl, *N*-furoyl and *N*-3,4,5-trimethoxybenzoylcyclobutanecarboxamides are moderately active tremorine antagonists. None of the compounds is active against pentylenetetrazole induced convulsions.

We have previously reported several imides and amides of cyclobutanecarboxylic acid[1–3] and cyclobutane-1,1-dicarboxylic acid[4,5] to have sedative and hypnotic properties. While studying imides of cyclobutanecarboxylic acid it was noticed that some were active antagonists to strychnine induced convulsions.[2] However, myorelaxant activity existed only in compounds with depressant effects. To better define the myorelaxant properties of cyclobutane containing compounds we have synthesized and evaluated the family of cyclobutanecarboximides in Table 1. The compounds have been examined for general central nervous system depressant properties, barbiturate potentiation, myorelaxant, antitremorine and anticonvulsant activity. The biological activity of these compounds has been empirically correlated with molecular structure and aqueous solubility.

## Experimental

*Chemical Methods.* The compounds were prepared using conventional routes involving amide acylation with cyclobutanecarbonyl chloride in either pyridine or *N*,*N*-dimethylformamide.[2] Analytical data and m.ps. have been reported earlier.[6]

*Pharmacological Methods.* In all bioassay procedures mice were used one time only. They were previously untreated with drug and permitted to feed *ad libidum*. Methods used to evaluate the compounds in Table 1 for sedative and hypnotic properties, barbiturate potentiation, myorelaxant, antitremorine and anticonvulsant potency were as described previously.[2]

## Discussion

To better elucidate structure activity relationships among the cyclobutanecarboximides the compounds in Table 1 were arranged according to structural and physiochemical properties. We had studied C-3 and C-4 cyclic imides of cyclobutanecarboxylic acid earlier and showed them to have some depressant activity.[1] In the present study both C-6 and C-10 cyclic derivatives, compounds 2 and 3, have been found inactive. The β-cyclopentylpropionyl derivative (1) is also inactive as a hypnotic and sedative. The lack of activity in these instances may be due to decreased solubility secondary to an increase of ring size and molecular weight. They were all insoluble in water. Their water solubility and that of the remaining compounds was observed when the synthesis mixtures were added to crushed ice.[2] Soluble products gave clear solutions; partially soluble or insoluble compounds yielded suspensions.

Factors other than water solubility also appear to play a part in the activity of these compounds. Introduction of α,β-unsaturation into the aliphatic chain of a compound with intermediate water solubility and activity, *N*-butyrylcyclobutanecarboxamide,[2] gave a water-soluble but inactive compound (5). However, introduction of a carbon–carbon double bond, did not consistantly give equivalent results. Introduction of unsaturation into the aliphatic chain of a slightly soluble and slightly active compound, *N*-isobutyrylcyclobutanecarboxamide,[2] gave a com-

*Part I: J. Med. Chem., **12**,923 (1969).

TABLE 1. BIOLOGICAL DATA FOR *N*-ACYLCYCLOBUTANECARBOXAMIDES.[a]

| No. | Acyl group | Gross effect[b] | R[c] | CNS[d] activity (category) | SLT[e] | |
|---|---|---|---|---|---|---|
| | | | | | Partial | Complete |
| 1. | β-Cyclopentylpropionyl | N | 0.8 | –[f] | 0/4 | 0/4 |
| 2. | Cyclohexanecarbonyl | N | 1.2 | — | 2/4 | 1/4 |
| 3. | 1-Adamantanecarbonyl | N | 1.9 | B | 0/4 | 0/4 |
| 4. | Methacrylyl | I | 3.1 | C | 3/4 | 3/4 |
| 5. | Crotonyl | N | 1.1 | — | 0/4 | 0/4 |
| 6. | 4-Hydroxybutyryl | (C)* | (2.1)[g] | C | (4/4) | (4/4) |
| | | I | 2.1 | — | 3/4 | 3/4 |
| 7. | Phenylacetyl | I | 3.0 | C | 3/4 | 2/4 |
| 8. | Chloroacetyl | D | 1.6 | A | 0/4 | 0/4 |
| 9. | Dichloroacetyl | S | 1.5 | A | 2/4 | 2/4 |
| 10. | 3,5-Dimethoxybenzoyl | N | 1.5 | — | 1/4 | 1/4 |
| 11. | 3-Methoxybenzoyl | N | 1.9 | B | 4/4 | 4/4 |
| 12. | 2-Methoxybenzoyl | N | 1.8 | B | 3/4 | 2/4 |
| 13. | Benzoyl | N | 1.4 | — | 3/4 | 2/4 |
| 14. | 2-Furoyl | S | 3.3 | C | 4/4 | 3/4 |
| 15. | 3,4,5-Trimethoxybenzoyl | I | 4.5 | C | 4/4 | 4/4 |
| | | N | — | — | (1/4) | (1/4) |
| 16. | 4-Methoxybenzoyl | N | 1.5 | — | 2/4 | 2/4 |

[a], In each test 1000 mg/kg (oral) was the applied dose, except where indicated. [b], S,I,M,C, slight, intermediate, marked and complete reduction in the spontaneous motor activity respectively. D, depression and death. [c], R is the ratio (drug + barbiturate sleep time)/(barbiturate sleep time + drug sleep time). Pentobarbital given intraperitoneally (50 mg/kg). [d], Central nervous system activity: A, central nervous system depressant ability only. B, barbiturate potentiation only. C, both central nervous system depressant and barbiturate potentiation ability. [e], Strychnine lethality test: strychnine sulphate, 2 mg/kg (intraperitoneal). This dose is 100% lethal. The mice die within 10–12 min. Partial: animals protected longer than 30 min. from death/animals tested. Complete: animals completely protected from death/animals tested. [f], The compound was not subjected to this test. [g], The dose used was 500 mg/kg (i.p).

pound (4) with similar solubility but increased activity. Thus, compounds 4 and 5 appear to be exceptions in the previously postulated theory that activity within this series parallels water solubility.[2] The exceptional behaviour may be due to differences in electron density around the CONHCO group and resonance involving the α,β-double bond and the carbonyl group. Whatever the reason, these two compounds show parameters in addition to water solubility may be activity determinants.

*N*-4-Hydroxybutyrylcyclobutanecarboxamide (6), in contrast to the non-hydroxylated analogous compound had good water solubility. When given orally in gum tragacanth solution, it was more potent than *N*-butyrylcyclobutanecarboxamide.[2] However, when administered intraperitoneally and in water solution a marked increase in activity was noticed. Under these conditions it was equipotent with *N*-formyl and *N*-acetylcyclobutanecarboxamides. From this it would appear that introduction of a hydroxyl group remote to the carbonyl system, renders the molecule more water-soluble and more active than the parent compound. This substitution appeared either to facilitate destruction in the gut or to impede uptake since oral administration was less effective than intraperitoneal.

Electron withdrawing groups usually decreased the activity of the parent compounds, probably by decreasing solubility. For example, compound 7 showed much less water solubility and activity than the parent *N*-acetylcyclobutanecarboxamide. Replacement of the *N*-acetyl group by *N*-chloro and *N*-dichloroacetyl groups, both of which decreased water solubility and electron density about the carbonyl system, gave interesting pharmacological results. The *N*-chloroacetyl derivative (8), first showed a slight depressant effect, but then produced central nervous system stimulation followed by death ($LD_{50}$ 1000 mg/kg). By contrast, the *N*-dichloroacetyl derivative (9), was only depressant in its effect. However, its activity was much lower than that of the parent compound.

The *N*-aroylcyclobutanecarboxamides 10–13, 15 and 16 were generally water-insoluble and inactive as central nervous system depressants. An outstanding exception is *N*-3,4,5-trimethoxybenzoylcyclobutanecarboxamide (15), which, though water-insoluble, showed some activity. This paralleled previous observations[7] that the 3,4,5-trimethoxybenzoyl moiety is a common component of various naturally occurring pharmacologically active compounds. The only heterocyclic imide studied, *N*-furoylcyclobutanecarboxamide, was very slightly soluble in water and accordingly showed slight central nervous system depressant activity.

To test our previous report[2] that the locus for barbiturate potentiation may be functionally different from sites involved in the directly measured depressant effect, a comparison was made of the barbiturate potentiation and general depressant effect of these compounds. It was presumed that if there were two functionally different locii for these activities we should be able to categorize these compounds according to three modes of central nervous system action, i.e. depression only (category A), potentiation only (category B), and depression plus potentiation

(category C). When analyzed for pentobarbital potentiation activity at a dose of 1000 mg/kg a sleep prolongation factor R (Table 1) was used as the criterion for effect. For compounds not causing loss of the righting reflex R is a measure of true potentiation and becomes significant when greater than 1.6.

As is apparent from Table 1 some compounds with hypnotic and sedative activity (4,6,7,14 and 15) also prolonged pentobarbital sleeping time significantly; they are in category C. Compound 6, when given orally (1000 mg/kg) did not prolong pentobarbital sleeping time, however, when given intraperitoneally (500 mg/kg) it was equipotent with *N*-acetylcyclobutanecarboxamide (500 mg/kg) as a barbiturate potentiator.[2] This supported the results of gross screening experiments. Compounds 8 and 9, active as hypnotics and sedatives, were found lacking in potentiator activity. They are in category A. Compounds 3, 11 and 12, found to be inactive during gross screening potentiated sleeping time significantly and consequently belong in category B. These observations lend further support to the hypothesis that two mechanistically separate modes of action exist for these compounds; one for barbiturate potentiation and other for depressant ability.

Myorelaxant activity of the meprobamate type, is conveniently studied by ascertaining whether a compound antagonizes strychnine lethality[8,9] In the present series of sixteen compounds twelve show myorelaxant activity whereas in the gross screening and barbiturate potentiation test only seven were active. This suggests that the compounds in question are predominantly myorelaxants and that hypnotic and sedative activity is a secondary effect. Further, these compounds can be classified into two categories. Some are only myorelaxant (2, 10–13 and 16) and belong to a class of compounds, the prototype example of which is mephenesin. Others show slight depressant effects alongwith myorelaxant activity (4,6,7,9,14 and 15), they belong to a class of compounds, the prototype example of which is meprobamate. At a dose of 1000 mg/kg (oral) compounds, 11 and 15, protected 100%, and three compounds (4,6 and 14) protected 75% of the test animals from strychnine lethality. The rest of the compounds (2,7,9, 10–12, 13 and 16) protected only 50–25% of the population tested. The five most active compounds those protecting 100–75% of the test population were tested intraperitoneally at 500 mg/kg; *N*-4-hydroxybutyrylcyclobutanecarboxamide (6), was found to yield 100% protection, the rest of the compounds were virtually inactive at this dose. Compounds 6 and 15 were further studied at 250 mg/kg (i.p.) and 500 mg/kg (oral) respectively. Both were found to protect 50% of the test animals from death. Compound 6, the most potent myorelaxant of the series, appears to be more potent than most of the clinically used strychnine antagonists[10,11] e.g. mephenesin, meprobamate (Table 2). When given orally it was much less active than when given intraperitoneally. This suggested poor uptake from gut or degradation. Compound 15 seems to be metabolically activated in the gut since if given intraperitoneally at 500 mg/kg if protected only 25% of the animals instead of 50% as when given orally.

TABLE 2

| Drug | Antistrychnine $ED^*_{50}$ (mg/kg) |
|---|---|
| Mephenesin[10,11] | 355 |
| Meprobamate[10,11] | 400 |
| Carisoprodol[10,11] | 980 |
| Compound 6 (i.p.) | 250 |
| Compound 15 (oral) | 500 |

*Antagonism of strychnine lethality in 50% of the animals tested.

Eight of the compounds (2–4,6,7,10,14 and 15) were also tested for anticonvulsant (pentylenetetrazole antagonism)[12] and antitremorine effects.[13,14] None showed antagonism to pentylenetetrazole-induced convulsions. However, five (4,7,10,14 and 15) moderately protected 80–20% of the test animals from tremorine-induced tremors at dose of 1000 mg/kg (oral).

**Acknowledgements.** This work was supported in part by the National Institute of Health, United States Public Health Service Research Grant NB-7548.

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