was added, and the flask was tightly closed and left at room temp. At the end of the required reaction time the solvent was removed under reduced pressure, and the product was purified as described in Table I.

Method C.—In a flask as above and using a condenser, *d*-pilocarpine (0.02 mole) was dissolved in Me_2CO (10 ml) with constant stirring while heating to about 50°. A freshly prepd soln of 0.025 mole of the halo organic reagent in 10 ml of Me_2CO was then slowly added and the mixt was heated to reflux for 30 min (reflux was not necessary for **9**). After cooling, the soln was transferred to a dry flask with 20 ml of dry Me_2CO and kept tightly closed at room temp for the required period. The Me_2CO was then removed under reduced pressure above a H_2O bath, and the residue was dried. For purification of the products see Table I. In the case of **23**, *d*-pilocarpine was added to a suspension of the halo org reagent in dry Me_2CO at reflux temp, and the product was collected by filtration.

Method D.—In a flask equipped as above, a soln of halo org reagent (0.02 mole) in 2-methoxyethanol (20 ml) was heated to 50° with stirring; then a soln of *d*-pilocarpine (0.02 mole in 25 ml of the same solvent) was added dropwise. The mixt was heated with stirring to 80° for 30 min and left tightly closed at room temp for the required period. The solvent was removed at 80° under reduced pressure, and the residue was dried over P_2O_5. For **4** no heating was required and the molar ratio was 1.5:1.

Whenever min amounts of the HBr of the pilocarpine were obtd as a side product, the sepn from the quaternary compds was accomplished from an aq soln at pH 7.5. The free pilocarpine was extd with $CHCl_3$ leaving the quaternary compd in the aq layer which was then lyophilized.

Compd 25 (**R** = *p*-$BrC_6H_5C_6HOHCH_2$).—3-(*N*-*p*-Bromophenacyl)-*d*-pilocarpinium bromide (**20**) (0.24 g, 5×10^{-4} mole) was dissolved in MeOH (30 ml), and $NaBH_4$ (0.12 g, 3×10^{-3} mole) was added and stirred for 1 hr. The soln was then filtered and adjusted to pH ~ 7 (with HCl 1:4). The solvent was removed under reduced pressure, and the residue was dried overnight in a desiccator over P_2O_5. This residue was then dissolved in a min quantity of abs EtOH and filtered. After evapn of the solvent, the yellowish product (0.29 g, 93%) was redissolved in abs EtOH, decolorized with activated C (Darco G 60; 15 min at 30°), and filtered, and the solvent was removed under reduced pressure above a water bath. The white product was stored over P_2O_5; ν_{max}^{film} 3450 (broad) and 1095 cm^{-1} (OH); $[\alpha]^{22}D$ + 21° (*c* 0.25).

Compd 26 (**R** = *p*-$C_6H_5C_6H_4CHOHCH_2$).—3-(*N*-*p*-Phenylphenacyl)-*d*-pilocarpinium bromide (**23**) (0.24 g, 5×10^{-4} mole) was dissolved in MeOH (20 ml) with stirring, and $NaBH_4$ (0.12 g, 3×10^{-3} mole) was added. The mixt was kept for 90 min at 30°, work-up as above. For the sepn and purifn, dry $CHCl_3$ was used producing a yellowish hygroscopic solid (0.22 g, 90%) which was stored in a desiccator over P_2O_5: ν_{max}^{film} 3450 (broad), and 1095 cm^{-1} (OH); $[\alpha]^{22}D$ + 21.6° (*c* 0.25).

Pharmacological Methods.—Anticholinergic activity was detd on a piece of guinea pig terminal ileum suspended in a 5-ml organ bath filled with Tyrode soln at 35°. Contractions were induced by 20 ng of ACh at intervals of 2 min. The height of the contraction was estimated before and during the presence of the test compd. In the case of **21** and **22** the concn was 1 μg, for **19** and **26** it was 2 μg, and for all other compds, 10 μg. Amounts refer to final concn in ml of bathing fluid. Antagonism was expressed as % redn of the control response to ACh, and represents the average of 2 assays in 2 separate prepns.

A number of compds were examined as substitutes to atropine in exptl organophosphate poisoning. Groups of 6 male mice of 20-g body weight were used. The test compds were injected ip at a preselected dose which did not cause observable abnormalities. This was immediately followed by adminstration of 40 mg/kg of pralidoxime methanesulfonate. Five min later, animals were injected sc with 3 × LD_{50} of tetraethyl pyrophosphate (TEPP). Solns of the materials were made up in saline immediately before use and injected at a max vol of 0.2 ml. Compds 20 and 23 were dissolved in propylene glycol and injected in a vol of 0.05 ml.

Acknowledgment.—We thank Professor A. Mahdelbaum from the Technion, Israel Institute of Technology, Haifa, Israel, for advice and discussions on the mass spectra.

Benzimidazo[2,1-*b*]quinazolin-12-ones. A New Class of Potent Immunosuppressive Compounds

W. H. W. LUNN,* R. W. HARPER, AND R. L. STONE

The Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana 46206

Received June 1, 1970

The benzimidazo[2,1-*b*]quinazolin-12-ones constitute a novel group of compounds, only the parent unsubstituted tetracyclic compound being known previously. They were prepared as part of a search for new immunosuppressive agents and have proven markedly active in the sheep erythrocyte antibody in mice (SEAM) assay. They are much more active than azathioprine in this assay.

Apart from establishing some of the general structural requirements for maximal immunosuppressive activity in benzimidazo[2,1-*b*]quinazolin-12-ones, we attempted to modify the *in vivo* transport of these compounds by making carrier derivatives. These derivatives were designed with a view to their undergoing cleavage *in vivo* to an active immunosuppressive component and an inactive carrier portion which served only to alter the *in vivo* distribution of the active component.

It is clear that the activity of a presumably reversible compound cannot be ascribed, with any certainty, to the fact that the expected cleavage did indeed precede the demonstrated activity. Immunosuppression may well have arisen from the intact molecule. Studies with labeled compounds would be required to distinguish between these two modes of action. As will be seen, there are results which are consistent with the idea that cleavage of the reversible carrier compounds is, at least in some instances, a prerequisite to immunosuppression.

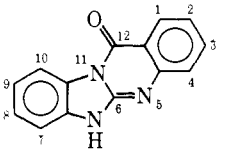
Details of the sheep erythrocyte antibody in mice (SEAM) assay used in these laboratories have been described elsewhere,[1] except to add that the dose levels used constituted the geometric series 50, 25, 12.5, 6.2, 3.1, 1.6. . . mg/kg. For better readability, the activities of the compounds are discussed throughout the paper rather than in the form of a single table. The number in parentheses next to a molecular diagram in the minimum dose, in mg/kg, which, when administered intraperitoneally at three optimal times (72, 48, and 24 hr

(1) C. J. Paget, K. Kisner, R. L. Stone, and D. C. DeLong, *J. Med. Chem.*, **12**, 1010 (1969).

prior to antigen), gives 4-fold or greater suppression of antibody response. The corresponding minimum effective doses of azathioprine and cortisone are 100 mg/kg (24, 48, and 72 hr after antigen) and 200 mg/kg (72, 48, and 24 hr prior to antigen), resp.

We now turn to structure–activity relationships, in particular the effects of substitution on the two aromatic rings (Table I). The parent member of the series (**1**) is

TABLE I

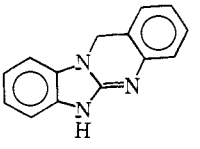


1

Compd	Substituent	MED, mg/kg
1		6.2
2	3-NO_2	12.5
3	(8 and 9)-Cl	6.2
4	7,8-Benzo	3.1
5	2-I	1.6
6	2,8,9-$(CH_3)_3$	0.8
7	3-CH_3	0.4
8	7,10-Cl_2-8,9-$(CH_3)_2$	0.4
9	8,9-$(CH_3)_2$	0.2
10	2,3-Benzo	0.1

active at 6.2 mg/kg; and while the nitro derivative **2** is less active, immunosuppression increases progressively from compound **3** to **10**. It is evident that substitution on the rings by hydrocarbon residues markedly enhances immunosuppression.

Removal of the carbonyl group results in a considerable reduction of activity, since the deoxy compound **11** is active only at doses of 25 mg/kg and above.



11

With respect to N-alkylation, it can be seen from the results below that in the *N*(6)-alkyl series the Me derivative is the most active. Furthermore, the *N*(5)-Me compound is much less effective than its N(6) isomer; and we have presumed this relationship of N(6)- to N(5)-substituted compounds is a general characteristic of this tetracyclic series. (Unless otherwise specified, all compounds discussed below are N(6) derivatives.) The compds, substituents, and MED (mg/kg) are as follows: **12**, CH_3, 1.6; **13**, C_2H_5, 3.1; **14**, *i*-C_3H_7, 6.2; **15**, C_3H_5, 6.2; **16**, C_5H_{11}, 3.1; **17**, C_8H_{17}, 12.5; **18**, 5-CH_3, 50.

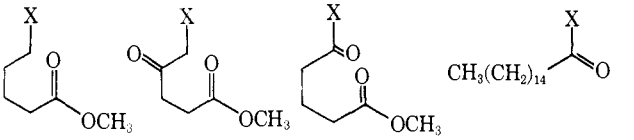
These benzimidazo[2,1-*b*]quinazolin-12-ones are generally *very* insoluble in H_2O, and the effect of imparting water solubility on a member is quite remarkable. Amine **19** is active at 6.2 mg/kg, while its water-soluble hydrochloride **20** is inactive at 50 mg/kg but toxic at 100 mg/kg. Similarly, the water-soluble potassium salt **22** does not have the activity of the corresponding acid **21** as can be seen in the following list of compds, substituents, and MED's (mg/kg) where X = **1**: **19**, X-$(CH_2)_3N(CH_3)_2$, 6.2; **20**, X-$(CH_2)_3NH(CH_3)_2Cl$, inactive at 50; **21**, X-CH_2COOH, 12.5; **22**, X-$CH_2COO^-K^+$, inactive at 50.

These data raise an important question. Does the very property of gross H_2O insolubility result in a specificity of the action? For example, the uptake of the H_2O-insoluble compounds in the particulate form by phagocytic cells might be expected to result in distribution parallel to that of antigen, whereas H_2O solubility could promote rapid excretion. This view is supported by subsequent investigations involving other routes of administration.[2] Alternatively, the alkyl substituents on the aromatic rings, leading to enhanced lipophilicity, might be postulated to promote distribution and/or cell penetration by means of interaction with lipid in tissue fluids or at cell surfaces.

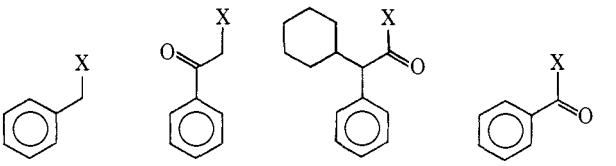
The parent tetracyclic compound **1** has a pK_a of 10.5. Since the anion of **1** is quite stable and should be capable of acting as a leaving group, it was hoped that the potentially reversible carrier derivatives made would cleave by either of the following mechanisms.

SNi SN2

Either mechanism could account for **24** and **25**, having much greater activity than **23** (with prior hydrolysis of the ester functions in the case of SNi mechanism). Furthermore, since longer alkyl chains at N (6) have been shown to reduce activity, the considerable activity of palmitoyl derivative **26** (again highly lipophilic) suggests prior SN2 hydrolysis, similar factors would, in part, account for the relative activities of **27**, **28**, **29**, and **30**.



23 (50) **24** (3.1) **25** (12.5) **26** (0.2)



27 (50) **28** (6.2) **29** (12.5) **30** (0.8)

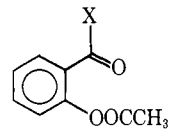
The *N*(6)-acyl and -aroyl compounds were found to be readily hydrolyzed. Their uv spectra were determined in neutral, acidic, and alk aq EtOH. In alk media, hydrolysis was so rapid that only the spectrum of the anion of **1** was generally observed. Acid-catalyzed hydrolysis was much slower, perhaps due to the stability of the protonated species.[3]

An indication of the relative importance of lipophilicity *vs. in vivo* hydrolytic reversal to **1** was obtained by determining the activities of aroyl analogs of **30**. The immunosuppressive activities of six of these analogs are given below in order of increasing activity.

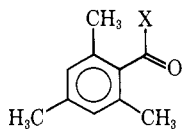
Uv studies revealed that **31**, **33**, and **35** behaved typically (they were rapidly hydrolyzed by alkali); but

(2) R. L. Stone, R. N. Wolfe, C. J. Paget, W. H. W. Lunn, and K. Gerzon, submitted for publication.

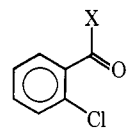
(3) W. H. W. Lunn and R. W. Harper, *Tetrahedron*, **27**, 2079 (1971).



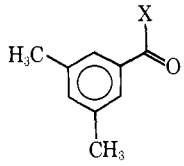
31 (3.1)



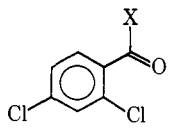
32 (3.1)



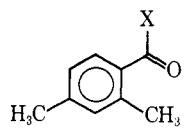
33 (0.8)



34 (0.4)



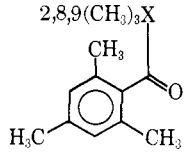
35 (0.4)



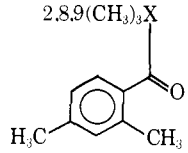
36 (0.2)

34, **36**, and particularly **32** (as might be expected on steric grounds) were hydrolyzed relatively slowly. It appears that the slower *in vivo* hydrolysis and/or increased lipophilicity of **34** and **36**, as compared to **30**, leads to increased activity. However, further reduction of the hydrolizability drastically reduced the immunosuppression, as seen with **32**.

A similar pattern is obtained with the methylbenzoyl derivatives **37** and **38** of the nucleus **6**.



37 (inactive at 3.1)



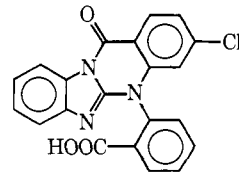
38 (0.1)

The relatively low activity of mesitoyl derivatives **32** and **37** may be due to the fact that the two *o*-Me groups sterically restrict the 2,4,6-trimethylbenzoyl function in a position where the plane of the benzoyl ring is approximately at right angles to the plane of the tetracyclic nucleus. Molecular models indicate that the angle between these 2 planes approximates to 90° in the *N*(6)-aroyl compounds. It could be argued that the benzoyl ring prevents close interaction of the benzimidazoquinazolone nucleus with a biochemical interface, and lessens the immunosuppression. If this were the case, then hydrolysis would be required for activity to be manifested.

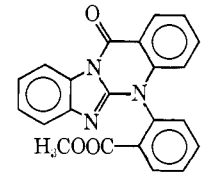
Compounds **39–41** are fairly inactive. It is known[4] that the "extranuclear" benzene ring in these compounds is also approximately at right angles to the tetracyclic nuclear plane though it will be remembered that the 5-Me compound is inactive.

We recognize the hazards of drawing rather detailed conclusions from biological results obtained with compounds which are generally *very* insoluble in H_2O.

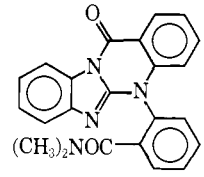
(4) W. H. W. Lunn and R. W. Harper, *J. Org. Chem.*, in press.



39 (inactive at 12.5)



40 (inactive at 12.5)



41 (inactive at 12.5)

However, all of the compounds discussed crystallize in the *same* form, namely very fine needles; and this *may* account in part for the consistency in relationship between structure and activity.

Regardless of the mechanisms involved, it is evident that these benzimidazoquinazolines are, in general, very active immunosuppressive compounds. Most of the compounds, by virtue of their high activity were administered only at low doses. However, the LD_0 values of **1** and **12** have been shown to be >1400 and >2000 mg/kg, and **4**, **5**, **7**, **8**, **9**, **13**, **19**, and **31** have been administered at 50 kg/mg with no deaths within 7 days These data would indicate that the therapeutic indices of the group as a whole is quite high. They are structurally very different from immunosuppressive compounds such as azathioprine, cortisone, cyclophosphamide, and methotrexate, and may serve to provide further insight into the complexities of the immune-response phenomenon.

Experimental Section

Apart from the two salts **20** and **23**, methods of preparg the compounds mentioned in this paper are described elsewhere.[5]

6-(3-Dimethylaminopropyl)benzimidazo[2,1-*b*]quinazolin-12-(6*H*)-one Hydrochloride (20).—The free amine (1.6 g) was stirred in *i*-PrOH (50 ml) while aq HCl (5.1 ml, 1 *N*) was added dropwise. The amine dissolved, and the mixt was evapd to dryness under reduced pressure. Addnl *i*-PrOH (80 ml) was added, and the mixt again was evapd to dryness under reduced pressure to remove traces of H_2O. The resulting solid was recrystd twice (MeOH–CCl_4); yield 1.18 g. *Anal.* ($C_{19}H_{20}ClN_4O$) C, H, N, Cl.

6-(Carboxymethyl)benzimidazo[2,1-*b*]quinazolin-12(6*H*)-one Potassium Salt (22).—The free acid (1.0 g) was stirred with KOH (0.23 g) in MeOH (8 ml) and H_2O (3 ml). The resulting soln was evapd to dryness under reduced pressure. *i*-PrOH (50 ml) was added to the residue, and the mixt was again evapd under reduced pressure. *Anal.* ($C_{16}H_{10}N_3O_3K$) C, H, N.

Acknowledgment.—We wish to thank Dr. Koert Gerzon, of these laboratories, for helpful discussions.

(5) W. H. W. Lunn and R. W. Harper, *J. Heterocycl. Chem.*, **8**, 141 (1971)