BIOLOGICAL ACTIVITY AND ACTIVE GROUPS OF NOVEL PYRAZOLES, THIOSEMICARBAZONES, AND SUBSTITUTED THIAZOLES

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A study of novel pyrazoles, thiosemicarbazones, and substituted thiazoles has shown a definite correlation between compound structure and antibacterial activity. Those compounds having a lipophilic chain had greater antibacterial activity than compounds having the less lipophilic structures such as the methoxy group. The hydrophilic core of the compounds contributes to movement of the compound into aqueous solution while the lipophilic characteristic enhances the ability to interact with the hydrophobic area of the membrane. Structural features that contribute to increased solubility and membrane interaction may greatly increase biological activity of compounds.

The cell membrane, which may function as a permeability barrier for the cell, plays an important role in antibacterial and anticancer chemotherapy. Regulation of nutrient passage through the barrier may contribute to a variety of known physiological states. Durham *et al*. (1) reported synthesis of unique heterocycles with functional groups which greatly increased solubility in water. The compounds demonstrated activity for both bacterial and tissue culture cells. Chesnut *et al.* (2) reported that a hydroxybenzindazole inhibited microbial growth and it was proposed that the compound produced a reversible distortion which disorganized but did not destroy the integrity of the membrane structure. This study extends the investigation of newly synthesized heterocycles, based on model steroids and compounds that have proven biological activity, in order to correlate biological activity with presence of certain functional groups. The greatest activity was found in especially designed pyrazoles, thiosemicarbazones, and related substituted thiazoles.

MATERIALS AND METHODS

The compounds were screened for growth inhibition against *Bacillus subtilis*, a gram-positive spore-forming rod, and *Pseudomonas fluorescens,* a gram-negative rod. The compounds (5 mg) were dissolved in 0.5 ml of dimethyl sulfoxide (DMSO). Sterile water was added to give a final volume of 5 ml. This stock solution (0.5 ml) was added to 4.5 ml of glucose minimal medium (3) to give a final concentration of 5 μg/ml for the test compound. Controls were run using sterile water and DMSO at the same concentrations as used with the test compound. The tubes were inoculated with an overnight culture of *B. subtilis* to an absorbancy of 0.04 at 540 nm. Growth was followed by measuring the change in absorbancy at 540 nm on a Coleman junior Spectrometer. The biological activity of the compound was determined by two measurements: (a) the change in absorbancy between 4 and 5 hours, which was determined from the linear part of the curve, and (b) the time required for the culture to reach an absorbance of 0.5. Compounds producing the smallest change in absorbance during the 1-hr interval and the longest time in reaching an absorbance of 0.5 were considered to have the greater biological activity.

RESULTS AND DISCUSSION

The growth of *B. subtilis* was measured in the presence and absence of 5 μg/ml of each test compound (Table 1). The control culture showed a change in absorbance of 0.170 between hours 4 and 5. The control required 5.5 hr to reach an absorbance of 0.5. The test compounds are listed in order of decreasing activity (Table 1). Compounds 1—5 (Figure 1) show the greatest biological activity. Compound 1 is most active since the growth was completely inhibited during the entire incubation period. Compounds 2 and 3 show pronounced growth inhibition while compounds 4 and 5 were less active. Compounds 6 and 7 were determined to be of intermediate activity

while 8, 9, 10 and 11 (Figure 2) showed no activity under the conditions of this experiment. These results were confirmed by an experiment run at a concentration of 20 μg/ml.

The structures of the most active compounds are presented in Figure 1. Compounds 1, 3, 4, and 5 each have a hydrophilic core and a lipophilic end (long hydrocarbon chain). The hydrophilic core enhances solubility of the compound in aqueous solution while the lipophilic group presumably facilitates transport across the membrane. Compound 2 does not have a lipophilic chain but does have a perimeter of hydrocarbon groups which tend to confer the lipophilic trait. It is proposed that change in the level of lipophilicity of compound is reflected in a change in the level of biological activity. Substitution of a methoxy group for the *n*-hexyl group on compounds 1, 3, and 4 greatly decreased the lipophilicity and the resulting compounds show no biological activity.

Compound 5 is a primary amine which could be more reactive than the pyrazoles (compound 1) because delocalization of the electron pair on nitrogen is probably not as great. However, because of the nucleophilic character of the amino group, it may react with membrane components and entry of the compound into the cell may be retarded.

The difference in lipophilicity may be seen between compounds 3 (Figure 1) and 8 (Figure 2). These compounds have the same active thiosemicarbazone unit, but while compound 3 has the long lipophilic chain, compound 8 has three methoxy functions on ring A that greatly decrease the lipophilic potential. The thiosemicarbazone unit has been postulated to chelate metals and its inhibitory activity may be associated with disruption of the metals on the membrane surface (4).

Compounds 2, 5, 6, 7, and 9 (Fig. 1 and 2) have the same nucleus with different substituents on the rings. The difference in compounds 2 and 5 may be attributed to the highly reactive free amino group. This finding augments the results obtained with compounds 1 and 5. Compounds 2, 6, and 7 have similar structures. The difference in activity may lie in the electronic and steric properties of the substituents on the heterocyclic ring. The most active compound was the one with the phenyl substituent (compound 2).

The intermediate activity of compound 6 may be due to the free amino group, which could readily interact with membrane components, while in compound 7 the low level of activity may result from the unfavorable geometry of the allyl group substituted on the amino group concomitant with the loss of the highly lipophilic hydrocarbon chain. When the amino group

TABLE 1. *Growth of* B. subtilis *in the presence of pyrazoles, thiosemicarbazones, and substituted thiazoles (5μg/ml).*

Compound	Absorbance at 540 nm		Time (hr) to reach absorbance at 540 nm of 0.5
	4 hr	5 hr	
Control	0.250	0.420	5.5
1	0.020	0.016	—
2	0.020	0.015	20.4
3	0.025	0.030	11.4
4	0.105	0.200	7.2
5	0.075	0.165	7.0
6	0.200	0.320	6.2
7	0.150	0.300	6.2
8	0.210	0.380	5.6
9	0.250	0.430	5.5
10	0.250	0.450	5.5
11	0.250	0.480	5.5

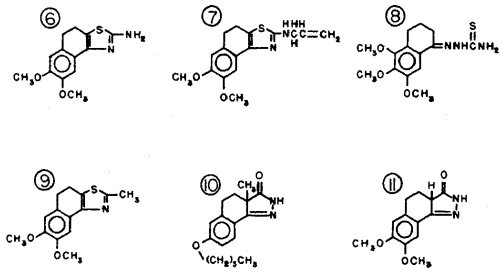

FIGURE 2. Pyrazolone, thiazole, and open-chain derivatives of 6-hexyloxy-1-tetralone, 6,7-dimethoxy-1-tetralone, and 5,6,7-trimethoxy-1-tetralone.

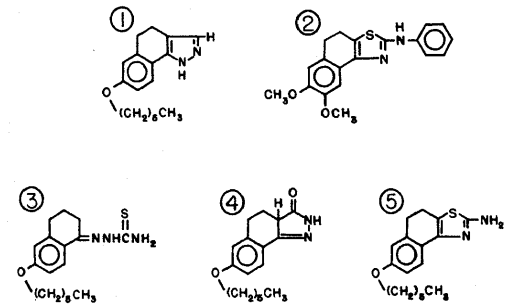

FIGURE 1. Pyrazole, pyrazolone, thiazole, and open-chain derivatives of 6-hexyloxy-1-tetralone and 6,7-dimethoxy-1-tetralone.

is replaced with a methyl group as in compound 9, there is total loss of activity.

Pyrazolones (compounds 4, 10, and 11) as a group did not exhibit much activity. Compound 4 was the most active which again may be due, in part, to the ability of the lipophilic chain to interact with cellular components and thus enhance transport of the pyrazolone to the active site. Compound 10 has a lipophilic chain but also has a bridgehead methyl which may be the cause of its loss of activity. In compound 11 the lipophilic chain has been replaced with the methoxy group and the bridgehead methyl removed.

CONCLUSION

In all likelihood, the compounds tested, like most antibiotics, have multiple sites of action. Obviously, two sites that may be involved are (a) the cell membrane and (b) inside the cell.

The biological activity of most of the compounds tested probably resides in the action of the heterocycle at some reactive site, with the exception of compound 3, which does not contain a heterocycle. The requirement for lipophilic groups can be nicely correlated with biological activity. All of the biologically active compounds show lipophilic traits. Possibly, these heterocycles may be more active because of the enhanced ability of the compound to penetrate the cell permeability barrier. It is postulated that the biological activity of compounds 1, 2, 3, 4, and 5 results from the improved mobility induced by the lipophilic groups. Compounds 8, 9, and 11 have less lipophilicity, show no biological activity, and possibly fail to enter the cell. Compound 10 does possess lipophilic potential but also has a bridgehead methyl which may interfere with the ability of the compound to interact with cellular components. Further studies are in progress to determine the mechanism of action of these compounds and the structure-activity relationships of such heterocycles.

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