DESIGN OF ANTINEOPLASTIC AGENTS ON THE BASIS OF THE "2-PHENYLNAPHTHALENE-TYPE" STRUCTURAL PATTERN. I. SYNTHESIS OF SUBSTITUTED 3-PHENYLQUINAZOLONES, BENZOXAZOLO[2,3-b]QUINAZOLONES AND BENZOTHIAZOLO[2,3-b]QUINAZOLONES

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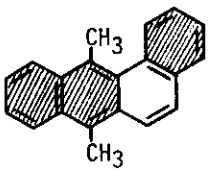
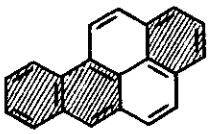
Abstract - A number of substituted 3-phenylquinazolin-4-ones, 12H-benzoxazolo-[2,3-b]quinazolin-12-ones and 12H-benzothiazolo[2,3-b]quinazolin-12-ones were designed and synthesized on the basis of a "2-phenylnaphthalene-type" structural pattern hypothesis. The postulated pattern, which was uncovered among a substantial number of compounds of both natural and synthetic origins, was noted to be associated with compounds possessing a variety of biological properties which include the antineoplastic activity. Several compounds designed for the present study were found to exhibit potent cytotoxicity against the growth of human promyelocytic leukemia (HL-60) cells.

On the basis of experimental information accumulated from previous work conducted in our laboratory and an exhaustive literature search, a tricyclic structural pattern consisting of a benzene ring attached to the 2-position of a naphthalene nucleus, or various heterocyclic ring components, was observed among a large number of biologically, pharmacologically and clinically active compounds of both natural and synthetic origins. [1] Activities of these compounds are extremely diverse. Examples of some of these compounds are depicted on the following page. For ease of recognition, the "2-phenylnaphthalene-type" structures are shaded.

DMBA (7,12-dimethylbenz[a]anthracene), benzo[a]pyrene and 5-methylchrysene[2] are potent carcinogens; coralyne[3] and nitidine[4] are active against leukemias P388 and L1210;[5] chartreusin (only structure of aglycone shown) and related antibiotics elsamicins A and B possess good antitumor activity as well as activities against both aerobic and anaerobic microorganisms;[6] a large group of isotetracene antibiotics (angucyclines), represented by rabelomycin,[7] are cytotoxic and active against Gram-positive bacteria. Many of these, such as aquayamycin, vineomycin, sakyomicin, etc., differ only in their degree of hydration or oxidation-reduction stage and some can be interconverted. The structure of these and two other groups of antibiotics, represented by the pluramycin (iyomycin) group antibiotic kidamycin[8] (which possess cytotoxic and antineoplastic action) and the anticoccidial antibiotic WS-5995A, [9] resemble that of benz[a]anthraquinone except that in the case of the pluramycins, the angular ring portion is a γ-pyrone and in the anticoccidial antibiotics, the unit linking the two parts is an α-pyrone. The alkaloids ellipticine[10] and 9-hydroxy-ellipticine,[11] and a synthetic pyridopyrrolo[2,3-g]isoquinoline derivative, BD-40,[12] show both antileukemic and anticarcinogenic activities. Flavoneacetic acid exhibits significant activity against colon adenocarcinoma 38, which is highly refractory to many established antitumor agents. It also has little or no major organ toxicity.[13] An interesting observation is that many flavones inhibit the aromatization of androstenedione and testosterone to estrogens (estrogen synthetase inhibitors)[14] but many antifungal isoflavones, such as genistein, are estrogenic.[15] An aza-isostere, methaqualone, is hypnotic, sedative and


DMBA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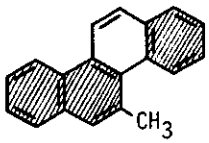
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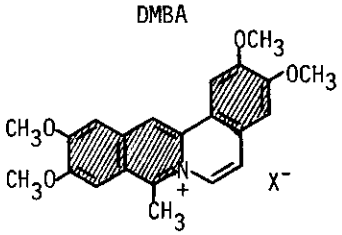
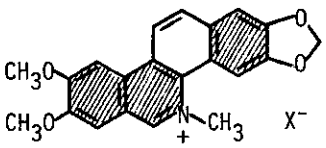
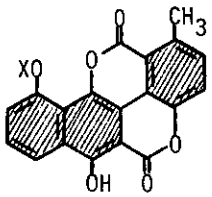
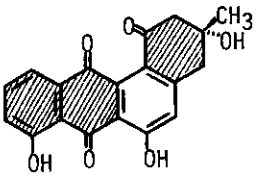
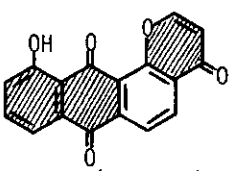
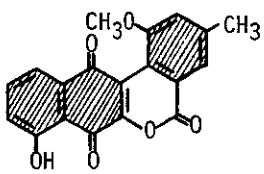

coralyne


nitidine


chartreusin (aglycone)


rabelomycin


kidamycin (chromophore)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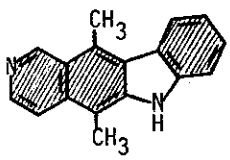
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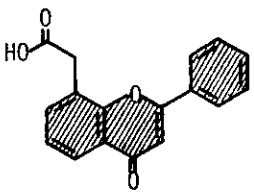
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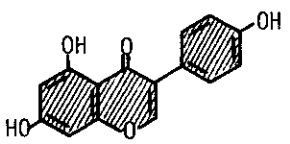
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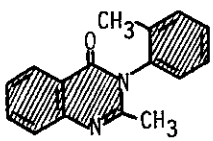
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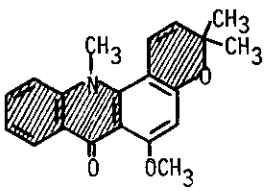
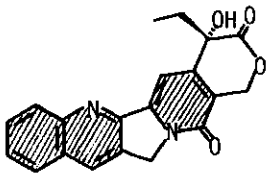
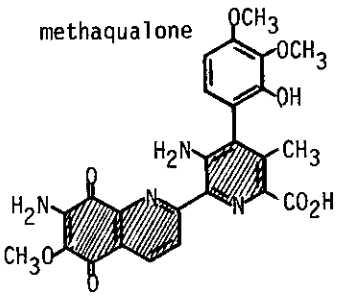
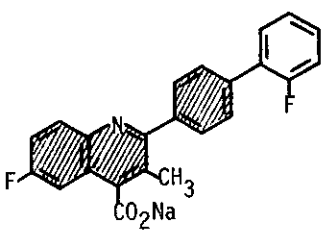
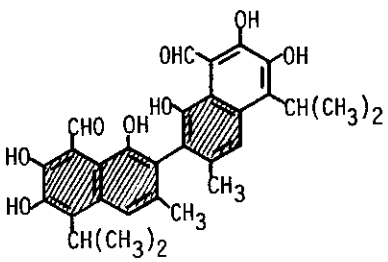

acronycine


camptothecin

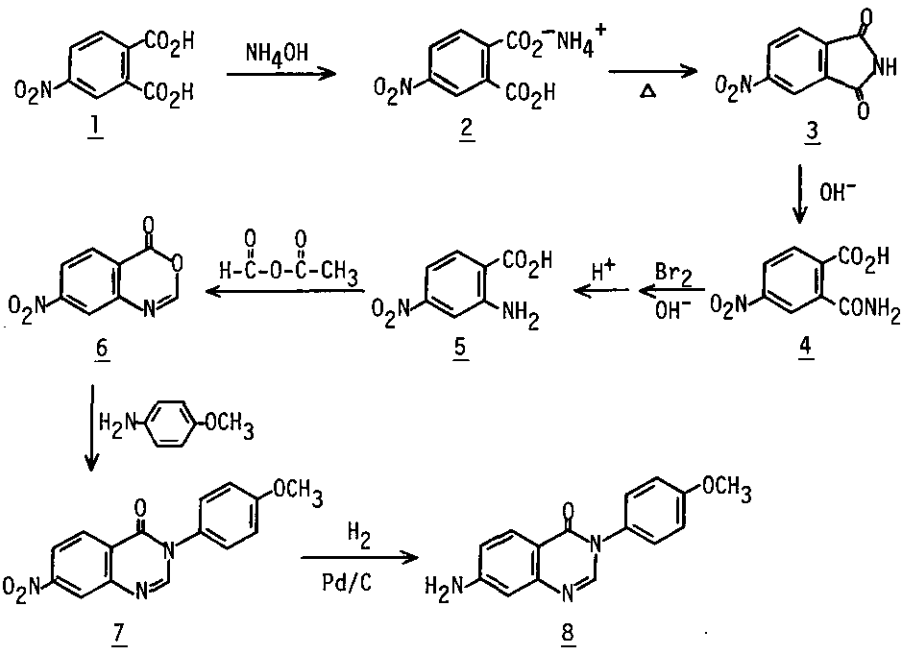

streptonigrin


DuP-785


gossypol

anticonvulsive.[16] Acronycine, an alkaloid acting primarily on membranous organelles,[17] reduces pain of the spine in patients with multiple myeloma.[18] The alkaloid camptothecin[19] and several hydroxylated derivatives are potent antileukemic agents.[20] A dark colored antibiotic streptonigrin[21] has demonstrated striking activity against a wide spectrum of experimental tumor systems.[22] Significant objective responses are detected with the methyl ester of streptonigrin in malignant lymphoma, mycosis fungoides, squamous cell carcinoma of the cervix, malignant melanoma, breast carcinoma and neoplasms of the head and neck.[23] A synthetic compound, DuP-785 (brequinar sodium), exhibits antileukemic activity and antitumor activity against human solid tumors in nude mice.[24] A symmetrically substituted carbocyclic compound gossypol, isolated from cotton, is an antioxidant with male contraceptive activity.[25] It has been found to be a specific inhibitor of DNA polymerase-α[26] and lactate dehydrogenase-X.[27]

The frequent occurrences of the 2-phenylnaphthalene-type molecular pattern among such a huge number of otherwise structurally unrelated natural and synthetic compounds with diverse biological activities suggest that this characteristic molecular rearrangement may be intimately related to the structures of certain pertinent biomolecules or may fit into some important bioreceptor sites which are of importance to the process of life. In order to test this hypothesis, a number of specifically designed compounds were prepared in our laboratory. This communication reports the synthesis of 3-phenylquinazolin-4-ones, 12H-benzoxazolo[2,3-b]quinazolin-12-ones and 12H-benzothiazolo[2,3-b]quinazolin-12-ones with chosen functional groups substituted at specific positions on these ring systems. The reason for initially choosing the 3-substituted quinolin-4-one ring system is that some compounds of this type, such as methaqualone and febrifugine, have exhibited a wide spectrum of biological properties including hypnotic, anticonvulsant, sedative, analgestic, antimicrobial, antimalarial, anthelmintic, antiinflammatory, antihistaminic, fungicidal and antitubercular activities. New compounds of this group for the present study were prepared by the route designed through a combination and modification of several reported synthetic steps.[28-33] As an example, synthesis of 7-amino-3-(4-methoxyphenyl)quinazolin-4-one (8) is described as follows:

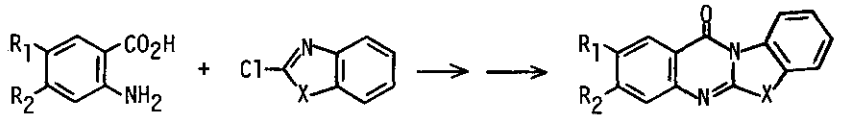


2-Aminocarbonyl-4-nitrobenzoic acid (4), prepared from 4-nitrophthalic acid (1) through the acid salt (2) and the imide (3), was treated with bromine and dilute aqueous potassium hydroxide at 0°C. The resulting Hofmann reaction product (5) was obtained in 95% yield, mp 232-234°C. Treatment of 5 with acetic-formic anhydride[34] followed by reacting the resulting 7-nitro-4-oxo-4H-3,1-benzoxazine (6) with p-anisidine in dry acetone with subsequent heating in dimethylformamide gave 3-(4-methoxyphenyl)-7-nitroquinazolin-4-one (7) in 39% yield, mp 255-256°C (from 1-butanol). The corresponding 7-amino derivative (8), mp 239-240°C, was obtained in 52% yield by catalytic hydrogenation of 7 with 10% palladium-on charcoal in dimethylformamide.

Other compounds prepared in a similar manner include 3-(4-methoxyphenyl)-5-nitroquinazolin-4-one (mp 193.5-194°C) and the corresponding 5-amino analog (mp 206-207°C), 5-nitro-3-phenylquinazolin-4-one (mp 178-180°C), 7-nitro-3-phenylquinazolin-4-one (mp 225-255.5°C), 3-(1,3-dimethoxyphenyl)-5-nitro-

quinazolin-4-one (mp 255-257°C) and 3-(4-chlorophenyl)-7-nitroquinazolin-4-one (mp 269.5-270°C). All intermediates and final products were properly characterized and verified by mass spectra determination and elemental analysis.

Since quite a few antineoplastic compounds containing the "2-phenylnaphthalene"-type structural feature carry a coplanar configuration,[1] for the present study, the phenyl ring and the quinazolinone moiety are also made coplanar by the addition of an ether or a thioether linkage. Consequently, several substituted tetracyclic benzoxazolo[2,3-b]quinazolinones and related benzothiazoloquinazolinones were designed. These compounds were synthesized by the following route based on the general procedure reported for their parent structures[35,36] with necessary reaction modifications.



9 + 10a X = O; b X = S → 11a R_1 = H; R_2 = NO_2; X = O

b R_1 = H; R_2 = NH_2; X = O

c R_1 = OH; R_2 = H; X = O

d R_1 = OH; R_2 = H; X = S

e R_1 = O-$(CH_2)_2$-$N(CH_3)_2$; R_2 = H; X = O

f R_1 = O-$(CH_2)_3$-$N(CH_3)_2$; R_2 = H; X = O

g R_1 = O-$(CH_2)_2$-$N(CH_3)_2$; R_2 = H; X = S

h R_1 = O-$(CH_2)_3$-$N(CH_3)_2$; R_2 = H; X = S

The synthesized compounds were evaluated for their cytotoxic effects of HL-60 (human promyelocytic leukemia) cells. The cytotoxicity of these compounds were determined by XTT-microculture tetrazolium assay.[37] The medium-effect inhibitory concentration (IC_{50}) was determined by the medium-effect plot [38] using computer software.[39] The IC_{50} values (μM) for the tetracyclic compounds are: 11a (>100), 11b (8.95), 11c (25.9), 11d (0.22), 11e (3.60), 11f (1.93), 11g (0.087) and 11h (0.13). The non-coplanar tricyclic compounds 7, 8 and related derivatives are not active in the HL-60 assays.

EXPERIMENTAL

Melting points were determined on a Thomas-Hoover apparatus. The ultraviolet spectra were measured on a Varian SuperScan 3 spectrophotometer. The mass spectra were determined at the KU mass spec laboratory, Lawrence, Kansas, and the elemental analyses were performed at the M-H-W Laboratories, Phoenix, Arizona.

2-Aminocarbonyl-4-nitrobenzoic Acid (4) - To 1000 ml of water containing 33g (0.58 mol) of KOH cooled at 0°C was added, with stirring, 50g (0.26 mol) of the imide (3, 98%). A light brown solution was formed in just a few minutes. It was stirred at room temperature for 4 h, then acidified by slow addition of 6N HCl with stirring. After about 80 ml of HCl was added, the solution became cloudy and soon white solids started to form. The mixture was stirred at 0°C for 90 min and filtered. The solid product was washed with a small amount of cold water and pressed as dry as possible during filtration. It was dried in vacuo for 3 days to give 54 g (98.7% yield) of 4, mp 148-150°C decomp. Anal. Calcd for $C_8H_6N_2O_5$: C, 45.72; H, 2.88; N, 13.33. Found: C, 45.51; H, 2.96; N, 13.10.

2-Amino-4-nitrobenzoic acid (5) - A mixture of 21 g (0.1 mol) of (4) and 100 ml of 1N KOH was stirred at 0°C. The solids did not dissolve completely. To the fine suspension was quickly added at 0°C, with rapid stirring, a mixture of 5 ml of bromine and 200 ml of 1N KOH followed by another 300 ml of 1N KOH. After brief stirring at 0°C, the mixture, which then became a light brown solution, was heated up immediately in a boiling water bath for 1 h. During that time, the color of the solution gradually turned into brick red. After which the stirred mixture was cooled at 0°C and was acidified with dropwise addition of 205 ml of 2N HCl. The color of the solution gradually faded to light orange with formation of solids accompanied by gas bubbles. This was particularly noticeable when about one-half amount of HCl had been added. Toward the end of addition, the reaction mixture was almost totally solidified and the appearance of the mixture resembled that of orange ice-crush. After 1 h stirring, the orange solid was collected by filtration and washed with cold water. It was purified by recrystallization from hot water to

give 17.3 g (95% yield) of orange needles, mp 234-234.5°C. Ms (m/z): 182(M^+). Anal. Calcd for $C_7H_6N_2O_4$: C, 46.16; H, 3.32; N, 15.38. Found: C, 46.18; H, 3.38; N, 15.35.

7-Nitro-4-oxo-4H-3,1-benzoxazine (6) - To 150 ml (1.6 mol) of Ac_2O cooled at 0°C was added dropwise, with stirring, 75 ml (1.9 mol) of 95% HCO_2H. After the addition was complete, the resulting solution was stirred at 0°C for 30 min then warmed between 50-55°C for 20 min. It was then cooled to 0°C. To the cold solution was added 9.1 g (0.05 mol) of 5. The solid gradually dispersed but a complete solution was not achieved. The mixture was heated at 50-55°C for 30 min and was allowed to stir at room temperature for 48 h. Still a complete solution was not formed. However, when the reaction mixture was evaporated by means of a rotatory evaporator at 40-50°C, a solution resulted (this interesting phenomenon was reobserved in repeated runs). The solution was exhaustively evaporated to yield a viscous semi-solid. This product (6) was not further purified but was used immediately for the preparation of 7.

3-(4-Methoxyphenyl)-7-nitroquinazoline-4-one (7) - To the residue from the previous run as added 100 ml of dry acetone. All solids dissolved. To the solution was added dropwise, with stirring, 7.4 g (0.06 mol) of p-anisidine in 50 ml of acetone. Heat was gradually evolved, but the amount of heat was so small that cooling was not necessary. White solid was formed almost immediately. The mixture was stirred for 3 h at room temperature and its volume was reduced to about one-half of the original. It was cooled at 0°C and filtered. The yellow solid collected had a mp of 234-235°C (decomp.).

To 100 ml of boiling dimethylformamide was added portionwise, with stirring, the aforementioned solid. A slight effervescence was noticed during each addition in the boiling mixture. The resulting solution was boiled for 90 min and the volume was reduced to 40 ml. A crystalline solid formed from the hot solution. The mixture was then cooled and was diluted with 150 ml of water. The resulting yellow solid was collected by filtration, washed with water, methanol and dried. Recrystallization from 1-BuOH yielded 5.8 g (39% overall yield from 5) of light yellow needles, mp 255-256°C; uv λ_{max}^{MeOH} nm (log ϵ): 208 (4.39), 232

(4.42), 327 (3.51). Ms (m/z): 297 (M^+). Anal. Calcd for $C_{15}H_{11}N_3O_4$: C, 60.61; H, 3.73; N, 14.14. Found: C, 60.69; H, 3.73; N, 13.97.

7-Amino-3-(4-methoxyphenyl)quinazolin-4-one (8) - A suspension of 5.9 g (0.02 mol) of 7 in 100 ml of dimethylformamide was hydrogenated at 1.7 kg/cm^2 in the presence of 0.6 g of 10% palladium-on-charcoal. Within 30 min theoretical amount of hydrogen was absorbed whereupon a solution was formed. The catalyst was removed by filtration and the filtrate was evaporated. The residue was purified by means of a Soxhlet extractor using ethanol as the solvent. After three days of the extraction process the ethanolic extract was evaporated. To the residue was added a small amount of 95% ethanol. The resulting reddish-yellow solids were collected by filtration and dried to give 2.8 g (52% yield) of 8, mp 239-240°C. Uv λ_{max}^{MeOH} nm (log ϵ): 201 (4.31), 258 (4.65), 285 (4.19). Ms (m/z) : 267 (M^+). Anal. Calcd for $C_{15}H_{13}N_3O_2 \cdot$ ¼ H_2O: C, 66.35; H, 4.92; N, 15.48. Found: C, 66.41; H, 5.07; N, 15.37.

3-Nitro-12H-benzoxazolo[2,3-b]quinazolin-12-one (11a) - To 290 ml of glacial AcOH was added at room temperature, with stirring, 10.1 g (0.055 mol) of 5. The solids did not dissolve completely. It was heated to 70°C and a solution was achieved. This was cooled to room temperature and to it was added dropwise 8.5 g (0.055 mol) of 2-chlorobenzoxazole (10a).[35] No heat was produced. The almost black mixture was refluxed with stirring for 4 h. During which time a brown solid gradually separated from the hot reaction mixture. Stirring was continued overnight at room temperature and the solid was collected by filtration. It was washed with methanol to give 8.6 g (61.3% yield) of 11a as golden crystals, mp 299-300°C (decomp.). Recrystallized from dimethylformamide yielded light golden crystals, mp 299-300°C (decomp.). Uv λ_{max}^{MeOH} nm (log ϵ): 207 (4.43), 234 (4.52), 362 (4.35). Ms (m/z): 281 (M^+). Anal. Calcd for $C_{14}H_7N_3O_4$: C, 59.79; H, 2.51; N, 14.94. Found: C, 59.99; H, 2.60; N, 14.90.

3-Amino-12H-benzoxazolo[2,3-b]quinazolin-12-one (11b) - To a golden brown suspension of 7.5 g of 11a and 80 ml of dimethylformamide was added 0.75 g of 5% palladium-on-charcoal. The mixture was hydrogenated at 2.5 kg/cm^2 until theoretical amount of hydrogen was absorbed (40 min). Catalyst was

removed by filtration and the dark brown filtrate was evaporated under reduced pressure. The resulting solid was recrystallized twice from dimethylformamide to give 3.1 g (46.3% yield) of 11b as brown crystals, mp >330°C. Uv λ^{MeOH}_{max} nm (log ϵ): 202 (4.22), 249 (4.64), 278 (4.28), 307 (4.27). Ms (m/z): 251 (M^+). Anal. Calcd for $C_{14}H_9N_3O_2 \cdot \frac{1}{2} H_2O$: C, 64.61; H, 3.87; N, 16.15. Found: C, 64.38; H, 4.06; N, 15.96.

2-Hydroxy-12H-benzoxazolo[2,3-b]quinazolin-12-one (11c) - To a suspension of 36.6 g (0.24 mol) of finely powdered 5-hydroxyanthranilic acid (9, R_1 = OH; R_2 = H) in 500 ml of glacial AcOH heated at 50°C was added dropwise 30.7 g (0.20 mol) of 10a with stirring. During the addition the grayish solid started to dissolve into the solution but soon a solid started to form from the dark brown mixture. The reaction mixture was refluxed for 6 h with stirring then was stirred continuously at room temperature overnight. The gray solid was collected by filtration and thoroughly washed with methanol until the filtrate appeared colorless and the solid product became light gray. Recrystallization from a mixture of dimethylformamide and methanol gave 22.1 g (43.8% yield) of 11c as off-white solids, mp 308°C. Uv λ^{MeOH}_{max} nm (log ϵ): 202 (4.17), 236 (4.70), 284 (4.41), 345 (3.76). Mx (m/z): 252 (M^+). Anal. Calcd for $C_{14}H_8N_2O_3$: 3, 66.66; H, 3.20; N, 1.11. Found: C, 66.41; H, 3.41; N, 10.86.

2-Hydroxy-12H-benzothiazolo[2,3-b]quinazolin-12-one (11d) - To a stirred mixture of 36 g (0.24 mol) of powdered 9 (R_1 = OH; R_2 = H) in 500 ml of glacial AcOH was added 45 g (0.27 mol) of 2-chloro-benzothiazole (10b). The mixture was stirred at room temperature for 10 min, then was heated to reflux, with continuous stirring. After about 90 min the stirring suspension, which was never dissolved, suddenly changed to a thick gray crystalline mixture which resembled a volcano formation with constant eruption. After about another 30 min the contents further changed to smaller suspending homogeneous articles and smooth stirring was resumed. The reaction mixture was heated and stirred continuously for another 4.5 h and cooled. The solids were collected by filtration and washed well with methanol and ether to give, after drying, 42.5 g of crude product. Recrystallization from a mixture of 1-BuOH and dimethylformamide

(1:1) gave 32.8 g (52% yield) of 11d as white crystals with silky luster, mp 312-313°C (decomp.). Uv λ_{max}^{MeOH} nm (log ϵ): 243 (4.64), 265 (4.29), 294 (4.33), 306 (4.30), 355 (3.83). Ms (m/z): 268 (M^+). Anal. Calcd for $C_{14}H_8N_2O_2S$: C, 62.67; H, 3.01; N, 10.44. Found: C, 62.43; H, 2.84; N, 10.39.

2-(2-Dimethylaminoethoxy)-12H-benzoxazolo[2,3-b]quinazolin-12-one (11e) - A mixture of 3.15 g (0.013 mol) of 11c, 20 ml of hexamethylphosphoramide and 50 ml of monoglyme was stirred at room temperature until a solution was obtained. To this solution was added 5.4 g (0.035 mol) of 2-dimethylaminoethyl chloride hydrochloride followed by dropwise addition of 5.2 g (0.038 mol) of K_2CO_3 in 10 ml of water. The addition took about 30 min. The resulting mixture was heated at 82-85°C for 1 h, then stirred overnight at room temperature. It was chilled to 0°C. The resulting precipitate was collected by filtration and washed with water to give 2.5 g of crude product. Recrystallization from ethanol gave 1.2 g (29.7% yield) of 11e as white crystals, mp 147-148°C. The base form of this product was converted to its hydrochloride salt in ethanolic HCl to give 0.95 g of pure compound, mp 266-268°C (ethanol). Ms (m/z): 323 (M^+). Anal. Calcd for $C_{18}H_{17}N_3O_3 \cdot$ HCl: C, 60.08; H, 5.04; N, 11.68. Found: C, 60.04; H, 5.21; N, 11.44.

2-(3-Dimethylaminopropoxy)-12H-benzoxazolo[2,3-b]quinazolin-12-one (11f) - This compound was prepared in a similar manner from 4 g (0.016 mol) of 11c, 10 g (0.063 mol) of 3-dimethylaminopropyl chloride hydrochloride and 11 g (0.08 mol) of K_2CO_3. Recrystallization from ethanol gave 2.05 g (38.3% yield) of the free base, mp 140-145°C (ethanol). The hydrochloride salt was prepared in ethanolic HCl, 1.85 g, mp 278-280°C. Ms (m/z): 337 (M^+). Anal. Calcd for $C_{19}H_{19}N_3O_3 \cdot$ HCl: C, 61.04; H, 5.39; N, 11.24. Found: C, 60.89; H, 5.56; N, 11.02.

2-(2-Dimethylaminoethoxy)-12H-benzothiazolo-[2,3-b]quinazolin-12-one (11g) - This compound was prepared in an analogous manner from 11d and 2-dimethylaminoethyl chloride in 30.7% yield , mp 169-170°C. Its hydrochloride salt melted at 277-278°C (ethanol). Ms (m/z): 339 (M^+). Anal. Calcd for $C_{18}H_{17}N_3O_2S \cdot$HCl: C, 57.52; H, 4.83; N, 11.18. Found: C, 57.78; H, 4.70; N, 10.96.

2-(3-Dimethylaminopropoxy)-12H-benzothiazolo[2,3-b]quinazolin-12-one (11h) - This compound was prepared in a similar manner from 6.7 g of 11d. The crude free base was converted directly to its hydrochloride salt to give 4.6 g (47.2% yield), mp 258-258.5°C (ethanol). Ms (m/z) : 353 (M^+). Anal. Calcd for $C_{19}H_{19}N_3O_2S \cdot HCl \cdot H_2O$: C, 55.96; H, 5.15; N, 10.30. Found: C, 55.86; H, 4.91; N, 10.09.

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