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Fused Pyrimidines as Potential Antimicrobic Agents

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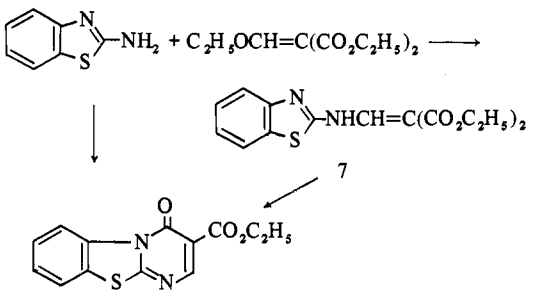
Amino heterocycles were condensed with ethoxymethylenemalonic ester (EMME) to form the corresponding heterocyclic aminomethylenemalonic ester. These esters were thermally cyclized to the next higher 3-carbethoxy-4-keto heterocycle. Cyclizations of the esters onto a ring nitrogen usually occurred except for naphthyridine formation from the corresponding pyridine. The intermediates and cyclic products were tested for antimicrobic activity *in vitro* and *in vivo* against M-240, Sa-27, and Ca-14 and they were inactive. When tested *in vitro* against Tr-25, compounds **17**, **18**, and **29** were active at 100 μg/ml.

This work was undertaken in order to provide heterocyclic compounds related to the urinary antiseptic nalidixic acid,[1,2] for screening as potential antimicrobial agents. Various heterocyclic amines were condensed with diethyl ethoxymethylenemalonate to form, in most cases, the corresponding enamine which was isolated and subsequently cyclized thermally to the desired product. Under certain conditions, the cyclic product could be formed without prior isolation of the enamine. The preparation of compound **24** will serve as an example of the reactions involved.



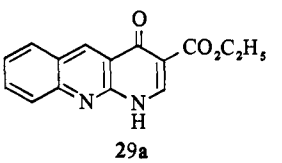
24

Syntheses of the enamines (Table I) were accomplished most readily by heating an equimolar mixture of the starting materials in the absence of solvent. Certain amines reacted immediately as evidenced by the evolution of heat upon mixing the reactants. To ensure a complete reaction, however, such mixtures were subsequently heated at steam bath temperature. As an exception to this procedure, 2-aminobenzimidazole yielded only the cyclic product (**27**) at 100, 25, or 5°.

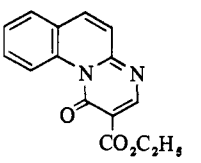
In three instances (compounds **21**, **22**, and **24**, Table II), cyclized products were prepared directly from the amine and diethyl ethoxymethylenemalonate in refluxing 1,2,4-trichlorobenzene (method B). For comparison, the corresponding enamines were cyclized under similar conditions. In the case of 2-aminobenzothiazole, the cyclic product (**24**) was prepared in better yield by the direct cyclization procedure. On the other hand, the cyclic product (**22**) derived from 2-amino-4-*p*-biphenylylthiazole was obtained in greatest yield by a cyclization of the enamine (**5**) intermediate. The direct condensation of 2-amino-5-nitrothiazole with diethyl ethoxymethylenemalonate formed a cyclic product (**21**) which was highly contaminated; thus, pure **21** could be obtained only by a cyclization of the enamine (**3**) intermediate. In view of these results, the cyclization of an enamine intermediate (method C) was preferred over the direct cyclization procedure (method B).

The enamine (**14**) obtained from 2-aminopyrimidine cyclized readily to the corresponding pyrimidopyrimidine (**30**) by method C but the enamine (**15**) from 2-amino-4,6-dimethylpyrimidine could not be cyclized under those conditions presumably due to steric hindrance of the methyl groups.

Of further interest were the cyclizations and attempted cyclizations of enamines **11**, **12**, **13**, **16**, **17**, and **18** according to the procedure described for the cyclization of **10** in refluxing diphenyl ether. It was not possible to cyclize **17** or **18** by this method, while **11** afforded an inseparable mixture. The formation of **28** or **31** from **12** or **16**, respectively, proceeded without difficulty. The cyclization of **13** afforded a product (**29**) whose melting point was unexpectedly low for the desired naphthyridine derivative. Previous work[7] has indicated that the enamines formed from 2-aminopyridines and diethyl ethoxymethylenemalonate at times cyclize to the corresponding pyridopyrimidine instead of the 1,8-naphthyridine. In view of this possibility the cyclization of **13** could yield **29a** or **29b** or both. The nmr spectrum of the product was measured in $CDCl_3$



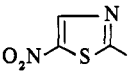
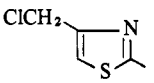
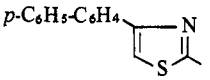
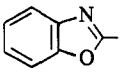
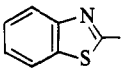
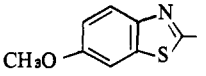
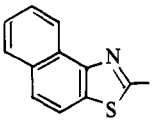
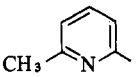
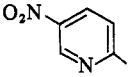
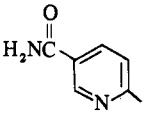
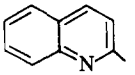
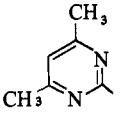
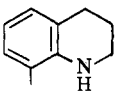
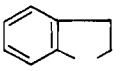
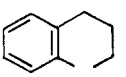
29a



29b

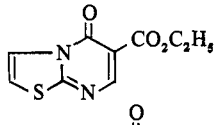
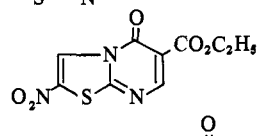
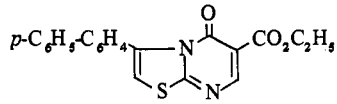
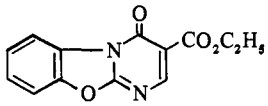
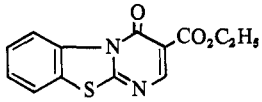
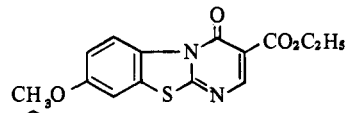
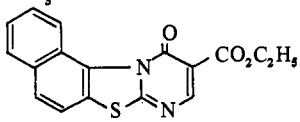
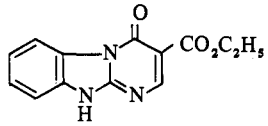
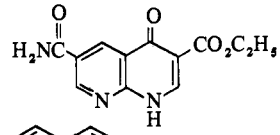
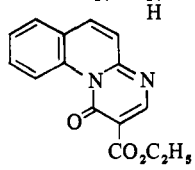
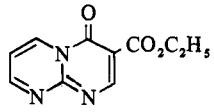
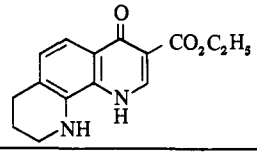
Table I. Enamines

$$\begin{matrix} R \\ R' \end{matrix}\!\!>\!NCH{=}C(CO_2C_2H_5)_2$$

No.	R	R'	Method[a]	Mp, °C	Yield, %	Formula	Recrystn solvent[b]
1		H	A, 50°, 0.5 hr	96–98	8	$C_{11}H_{14}N_2O_5$	E-PE
2		H	A, 125°, 1 hr	59–60[c]	69	$C_{11}H_{14}N_2O_4S$	PE
3		H	A, 145°, 1 hr	98–100	50	$C_{11}H_{13}N_3O_6S$	PE
4		H	A, 150°, 1 hr	95–96	8	$C_{12}H_{15}ClN_2O_4S$	Et
5		H	A, 150°, 1 hr	149–150	50	$C_{23}H_{22}N_2O_4S$	B-PE
6		H	A, 115°, 1 hr	106–108	58	$C_{15}H_{16}N_2O_5$	Et
7		H	A, 100°, 2 hr	105–106	85	$C_{15}H_{16}N_2O_4S$	PE
8		H	A, 100°, 1 hr	145–146	51	$C_{16}H_{18}N_2O_5S$	Et
9		H	A, 125°, 0.5 hr	158–159	82	$C_{19}H_{18}N_2O_4S$	Et
10		H	B	107.0–107.5[d]	30	$C_{14}H_{18}N_2O_4$	PE
11		H	A, 165°, 0.25 hr	169–170	30	$C_{13}H_{15}N_3O_6$	Et
12		H	A, 145°, 1 hr	198–199	44	$C_{14}H_{17}N_3O_5$	DMF
13		H	A, 100°, 2 hr	115–116[e]	87	$C_{17}H_{18}N_2O_4$	Et
14		H	A, 135°, 1 hr	115–117[f]	59	$C_{12}H_{15}N_3O_4$	PE
15		H	A, 135°, 1 hr	77–78	30	$C_{14}H_{19}N_3O_4$	PE
16[g]		H	A, 100°, 1.5 hr	146.5–148.0	87	$C_{17}H_{22}N_2O_4$	Et
17			A, 100°, 8 hr	41–43	38	$C_{16}H_{19}NO_4$	Et
18			A, 100°, 6 hr	208–209 (2.0 mm)[h]	59	$C_{17}H_{21}NO_4$	
19			A, 100°, 5 hr	198–200 (2.0 mm)[h]	79	$C_{16}H_{25}NO_4$	

[a] Methods are described in the Experimental Section. All compounds analyzed within ±0.4% of theoretical for C, H, and N. [b] A, AcOH; B, C_6H_6; DMF; E, Et_2O; Et, EtOH; PE, petr ether. [c] Ref 3 gives mp 60–61°. [d] Ref 4 gives mp 113–114°. [e] Ref 5 gives mp 118°. [f] Ref 6 gives mp 113°. [g] A picrate formed (mp 226–227°) but treatment of 16 with HONO followed by alkaline β-naphthol gave no coupling product, thus indicating substitution at the 8-amino position. [h] Boiling point.

Table II. Cyclized Products

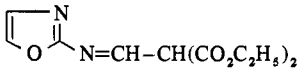
No.	Structure	Method[a]	Mp, °C	Yield, %	Formula	Recrystn solvent[b]
20		C	184–185[c]	78	$C_9H_8N_2O_3S$	Et
21		C	227–228	33	$C_9H_7N_3O_5S$	B
22		C B	165–166[d]	53 29	$C_{21}H_{16}N_2O_3S$	Et
23		C	154–155 dec	80	$C_{13}H_{10}N_2O_4$	Et
24		B C	142.5–143.5 143.5–144.0	49 28	$C_{13}H_{10}N_2O_3S$	Et
25		C	191–192	76	$C_{14}H_{12}N_2O_4S$	Et
26		C	158.5–160.0	63	$C_{17}H_{12}N_2O_3S$	Et
27		A, 100°, 5 min	286–287 dec[e]	27	$C_{13}H_{11}N_3O_3$	A
28		D	260–261	48	$C_{12}H_{11}N_3O_4$	DMF
29		D	91–92	48	$C_{15}H_{12}N_2O_3$	PE
30		C	135.0–136.5	19	$C_{10}H_9N_3O_3$	B
31		D	250–252 dec	2.3	$C_{15}H_{16}N_2O_3$	Et

[a]Methods are given in the Experimental Section. All compounds analyzed within ±0.4% of theoretical for C, H, and N. [b]See footnote *b*, Table I. [c]Ref 8 gives mp 186°. [d]Ref 8 gives mp 169°. [e]Ref 9 gives mp 270–271°.

with TMS as an internal standard. A spectrum, taken after shaking the solution with NaOD-D_2O, remained unchanged. The low melting point of the product and the absence of exchangeable protons favor **29b** as the structure. The nmr spectrum of **29** exhibited a triplet at δ 1.43 and a quartet at 4.43 which have been assigned to the carbethoxy protons. A singlet at 8.75, integrating for one proton, has been assigned to the proton at the 3 position of the 2-carbethoxy-1*H*-pyrimido[1,2-*a*]quinolin-1-one. A complex pattern, centering on δ 9.65 and integrating for one proton, has been assigned to the proton at the 10 position, by analogy with the recorded[10] chemical shift of the proton at the 8 position in quinolines. The remaining peaks include a pair of doublets (J = 9 Hz) integrating for one proton each at δ 7.30 and 7.90, and a complex pattern under another doublet (J = 6.6 Hz) at 7.59. The latter may be due to the vinyl proton at the 5 position. An analogous reaction has been reported by Antaki and Petrow[11] who condensed 2-chloroquinoline with ethyl β-aminocrotonate to form 1*H*-3-methylpyrimido[1,2-*a*]quinolin-1-one.

The electronic absorption maxima and the location of the carbonyl bands[12] in the infrared spectra of the enamines

and of the cyclic compounds are listed in Table III.† The infrared spectrum of diethyl ethoxymethylenemalonate exhibited a broad ester carbonyl band at 5.84 μ while the spectra of most of the enamines exhibited carbonyl absorption in the vicinity of 5.9 μ indicating conjugation between the enamine nitrogen and the carbonyl groups. The spectrum of **1**, however, exhibited a carbonyl band at 5.78 μ which is the region in which diethyl malonate and other saturated esters absorb. These data indicate that **1** may exist in the imine form (**1a**).



1a

Biological Screening Results. The compounds of Tables I and II (except **1** and **27**) were screened *in vitro vs. Staphylococcus aureus* (M240), *Salmonella schottmuelleri* (Sa-27), *Candida albicans* (Ca-14), and *Trichophyton mentagrophytes* (Tr-25) at 10, 30, and 100 μg/ml. Compounds **17**, **18**, and **29** were active against Tr-25 at 100 μg/ml. All of the compounds except **31** were also tested *in vivo* against M-240, Sa-27, and Ca-14 and were found to be inactive. Compound **31** was tested *in vivo* against Sa-27 and was inactive.

Other screening systems employed include sarcoma 180 (mice), polio II, and APC I (tissue culture), in which these compounds were inactive. The compounds were also ineffective against mice challenged intranasally with 1000 LD_{50} of influenza PR-8 virus and were ineffective in reducing lung consolidation of mice challenged intranasally with 10,000 LD_{50} of influenza PR-8 virus.

Experimental Section

All melting points were taken on a calibrated Thomas-Hoover melting point apparatus. The yields reported in Tables I and II represent the yields of analytically pure products. The ir spectra were measured on a Perkin-Elmer Model 21 spectrophotometer using KBr disks except in the case of liquid products whose spectra were measured neat. The uv absorption spectra were measured on a Cary ultraviolet spectrophotometer using MeOH solutions except as otherwise noted in Table III. The 8-amino-1,2,3,4-tetrahydroquinoline,[13] 2-aminooxazole,[14] and 2-aminobenzoxazole[15] were prepared according to methods cited in the literature. The other amines which were employed as starting materials were obtained commercially.

General Procedures. Method A. Equimolar amounts of diethyl ethoxymethylenemalonate (EMME, Gallard-Schlessinger) and the amine were heated under the conditions indicated (see Tables I and II). The reaction mixture was then cooled to room temperature. Solid products were filtered and recrystallized from the appropriate solvent while liquid products were purified by vacuum distillation. Et_3N and an equivalent of 2-amino-4-chloromethylthiazole hydrochloride were employed in this procedure in order to obtain compound **4**.

Method B. Diethyl ethoxymethylenemalonate was dissolved in 4–8 times its weight of 1,2,4-trichlorobenzene. An equimolar amount of the amine was added and the solution was heated. The ethanol which formed was fractionally distilled and collected in a Dean–Stark tube. When the theoretical amount of ethanol had been collected or when the ethanol ceased to evolve, heating was discontinued. The cooled solution was diluted with three volumes of petroleum ether (bp 75–90°), and the product which separated was recrystallized from the appropriate solvent.

Method C. The appropriate enamine was dissolved in seven times its weight of 1,2,4-trichlorobenzene and treated according to method B.

Method D. The appropriate enamine was added in small portions, over a 10-min period, to about 15 times its weight of refluxing diphenyl ether, whereupon vigorous effervescence occurred. The solution was refluxed for 2 hr after the last addition of enamine. It was cooled and diluted with three volumes of petroleum ether (bp 75–90°). The product which separated was then recrystallized from the appropriate solvent.

References

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†Supplementary material giving infrared and ultraviolet absorption data on the described compounds (Table III) will appear immediately following this article in the microfilm edition of this volume of the journal. Single copies may be obtained from the Business Operations Office, Books and Journals Division, American Chemical Society, 1155 Sixteenth Street, N.W., Washington, D. C. 20036. Remit check or money order for $3.00 for photocopy or $2.00 for microfiche, referring to code number JMED-72-1203.