CHEMOTHERAPY OF BACTERIAL INFECTIONS

Part III. Synthesis of (N^4)-Amino-substituted Heterocyclic Derivatives of Sulphanilamide

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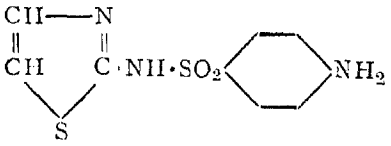
The synthesis of 2-N^1-sulphanilamidothiazol (I) and also its striking therapeutic properties in experimental plague, *streptococcal* (*hemolytic*) and *pneumococcal* infections in mice have been reported from this Institute.[1–5] The efficacy of this new drug in the other types of *pneumococcal*, *meningococcal*, *staphylococcal* and *lymphogranuloma venerum* infections has been described by many American workers.[6] There are also some preliminary reports of clinical trials of this and the related 4-methyl derivative in *pneumococcal* and *staphylococcal* infections.[7] Very significant of all these are the findings of Sokhey and Dikshit[8] that this new drug shows a striking specific therapeutic effect in plague infections far superior to 2-sulphanilamidopyridine. As a natural sequel to this important discovery, the synthesis of similar and related types of heterocyclic derivatives of sulphanilamide were undertaken with a view to giving an extensive trial to these promising classes of compounds.

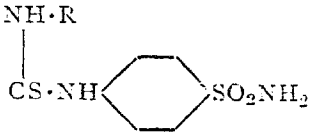
Heterocyclic derivatives of sulphanilamide can in general be of two classes according as the heterocyclic ring is substituted in the amino (N^4) or the sulphonamido (N^1) radical of sulphanilamide. It has been pointed out[1, 2] that whereas the derivatives of the first group may either be of poor activity or possess no advantage over sulphanilamide, only those of the second group may be more active and polyvalent in action than the parent sulphanilamide. The object, however, in deliberately undertaking at present the synthesis of heterocyclic derivatives of the first (N^4-amino substituted) group is (1) to verify the above conclusion and (2) to examine, in case some of them prove to be quite active, whether the toxic reactions, now associated with the free amino group of sulphanilamide and its derivatives,[9] are less intense or absent in them. In this paper, we report the synthesis of N^4-amino substituted thiazol, thiazolin, pseudothiohydantoin and acridine derivatives of

sulphanilamide undertaken with the above objective; the corresponding (N^1) sulphonamide substituted isomers and derivatives will be described in a future communication.

Sulphanilamide hydrochloride reacted with potassium thiocyanate to furnish N-*para*sulphonamidobenzene-thiocarbamide (II, R–H). On treating with 1 : 2-dichloroether the thiourea derivative yielded a thiazol which, by analogy, can be represented as 2-*para*sulphonamidobenzene-aminothiazol (III, $R' = R'' = H$) or its desmotropic form 2-*para*sulphonamidobenzene-iminothiazolin (IV, $R = R' = R'' = H$). On heating the above thiourea



(I)



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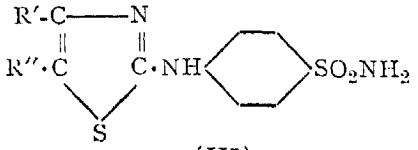
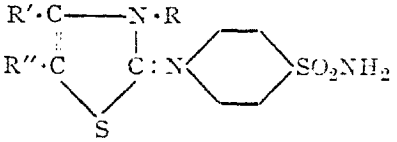


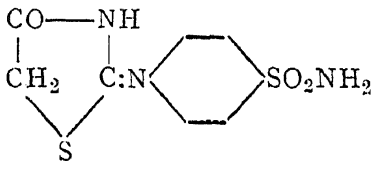
(III)



(IV)

derivative with phenacylbromide, the corresponding phenylthiazol derivative (III, $R' = Ph$, $R'' = H$) capable of existing in the desmotropic form (IV, $R = R'' = H$, $R' = Ph$) was produced. Similarly, N-*para*sulphonamidophenyl-N′-phenyl thiocarbamide (II, $R = Ph$)[1] and N-*para*sulphonamidophenyl-N′-allylthiocarbamide (II, R = allyl)[10] with 1 : 2-dichloroether yielded respectively 2-*para*sulphonamidobenzeneimino-3-phenylthiazolin (IV, $R = Ph$, $R' = R'' = H$) and 2-*para*sulphon-amidobenzeneimino-3-allylthiazolin (IV, R = allyl, $R' = R'' = H$). The allylthiocarbamide derivative (II, R = allyl) with phenacyclobromide furnished 2-*para*sulphonamido benzeneimino-3-allyl-4-phenylthiazolin (IV, R = allyl, R' = phenyl, $R'' = H$). Ethyl-α-bromoacetoacetate, ethyl-γ-bromoacetoacetate and ethyl-β-bromolevulinate condensed with N-*para*sulphonamidophenylthiocarbamide to yield respectively ethyl-2-*para*sulphonamidobenzeneamino-4-methylthiazol-5-carboxylate (III, $R' = CH_3$, $R'' = CO_2Et$), ethyl-2-*para*sulphonamidobenzeneaminothiazolyl-4-acetate (III, $R' = -CH_2CO_2Et$, $R'' = H$) and ethyl-2-*para*sulphonamidobenzeneamino-4-methylthiazolyl-5-acetate (III, $R' = CH_3$, $R'' = -CH_2CO_2Et$), these compounds also being capable of existing in the corresponding desmotropic forms of formula (IV). In all the above condensations, formation of thiazol or thiazolin derivatives with the *para*sulphonamidobenzene group in position 2 in preference to the position 3 is assumed only by analogy and not with any rigorous experimental proof.

Chloracetic acid, chloraceticester or chloracetylchloride condensed with N-*para*sulphonamidophenylthiocarbamide and the compound obtained can be either N^2-*para*sulphonamidophenylpseudothiohydantoin (V or VI) or 3-*para*sulphonamidophenylpseudothiohydantoin (VII).* By analogy with the investigations of previous workers,[11] we prefer the structure (V or (VI) for this compound. Adams, Long and Jeanes[12] by treating *para*sulphonamidochloracetanilide with ammonium thiocyanate obtained a product for



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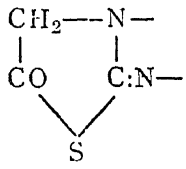
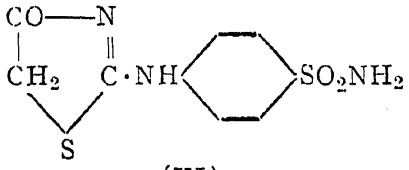
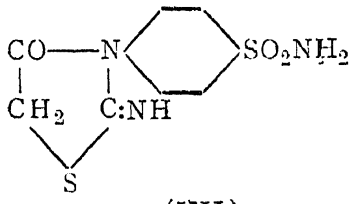


(Va)

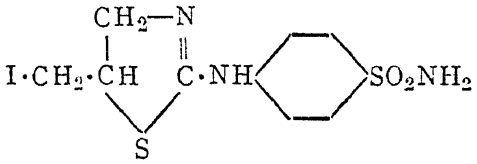


(VI)

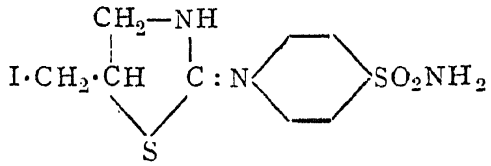


(VII)

which they have assigned the structure (VII) though there are instances in literature[13] to indicate that this compound can possess the alternative structure (V) or (VI) as well. We are not sure whether our product is identical with that of Adams *et al.* but it is of interest to note that in our experiments we could isolate some sulphanilamide evidently formed by the hydrolysis of the compound of structure (V). N-allyl-N′-*para*sulphonamidobenzenethiocarbamide on treating with iodine in alcoholic solution yielded a heterocyclic derivative which by analogy can be represented as 2-*para*sulphonamidobenzene amino-5-iodomethyl Δ^2-thiazolin (VIII) or the desmotropic thiazolidon (IX)



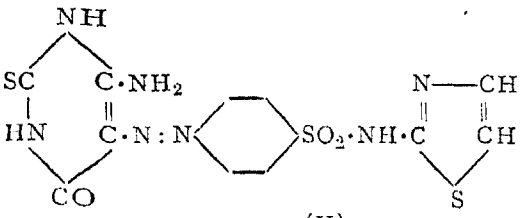
(VIII)



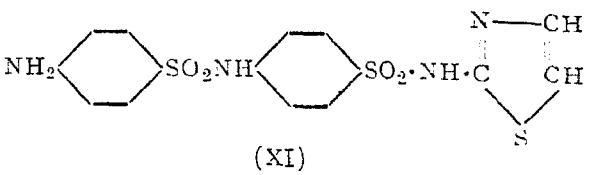
(IX)

2-Sulphanilamidothiazol (I) on being diazotised and coupled with 4-aminothiouracil yielded the dye (X); on being condensed with *para*acetaminobenzenesulphochloride and the product hydrolysed, the derivative (XI

* In this reaction, compounds of the isomeric type (Va) do not appear to be formed. The few instances in older literature wherein such a structure has been assigned to compounds obtained by treating chloracetic acid with thiourea derivatives, should be revised. Andreasch[14] obtained a "thiohydantoin" (now to be called pseudothiohydantoin) derivative by a similar method and assigned it the now accepted structure but in Beilstein's Handbuck (Bd. XXVII, Syst. No. 4[illegible] p. 239) it is wrongly recorded to be of type (Va).



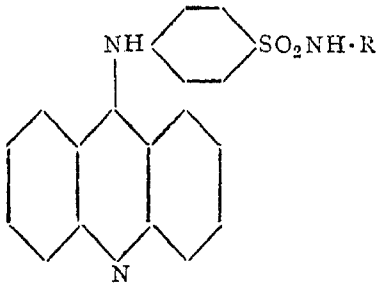
(X)



(XI)

was obtained. The gold salt of the derivative (X) and similar ones reported previously,[1] are for trial in tuberculosis.

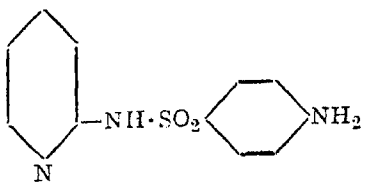
Five typical N^4-substituted sulphanilamide derivatives of acridine of formula (XII) have been synthesised by condensing 9-chloracridine with sulphanilamide, 4-sulphanilamidobenzenesulphonamide, 4′ : 4-diamino-benzenesulphoanilide, 4-amino-4′-nitrobenzenesulphonanilide and 2-sulphanilamidopyridine.



(XII)

All the thiazol, thiazolin and pseudothiohydantoin derivatives described were found to be inactive in *streptococcal* and *pneumococcal* infections in mice. The two derivatives (X) and (XI) were also inactive in *pneumococcal* infections; but they showed considerable activity in *streptococcal* infections. None of the acridine derivatives showed any *antipneumococcal* activity but a few showed considerable *antistreptococcal* activity. (The details of the animal experiments carried out in this Institute will be published elsewhere.)

The above results lend additional support to the view that for pronounced antibacterial action the heterocyclic ring should be substituted in the sulphonamide radical of sulphanilamide with the amino group being free.[1] It is thus very significant that whereas 2-sulphanilamido-thiazol (I) and 2-sulphanilamidopyridine (XIII) possess remarkable antibacterial action, the isomeric derivatives (III, $R' = R'' = H$) and (XIV)[15] are inactive.



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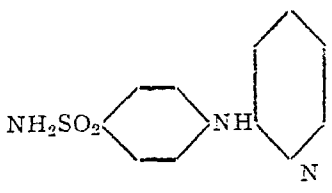

(XIV)

The activity of the (N^4) amino-substituted derivatives of sulphanilamide reported till now can be traced to the sulphanilamide liberated from them *in vivo*.[1,16] The only doubtful cases is that of *para*sulphonamidophenylguanidine[17]; it is not clear whether this compound also yields sulphanilamide *in vivo* or whether the guanidine radical itself, now known to be of much significance in the chemotherapy of protozoal infections,[18] possesses some inherent therapeutic property.

It is of course tempting to suggest that the importance of the free amino group as indicated above may have some bearing on the hypotheses postulated that the activity of sulphanilamide (and possibly the other derivatives also) is due to the biologically mediated oxidation or oxidation-reduction systems involving the free amino group.[19] But very recently it has been shown that the action of sulphanilamide is due to the competitive inhibition of some vital enzyme reaction of the bacterial cell which has for its substrate the "essential metabolite", *para*aminobenzoic acid.[21] Since the inhibitory action of sulphanilamide is due to its structural similarity to the *para*aminobenzoic acid, the importance of a free amino group in the sulphanilamide derivatives is understandable.

Experimental

N-para*sulphonamidobenzenethiocarbamide* (*II*, $R = H$).—*Para*aminobenzenesulphonamide (110 g.) dissolved in water (500 c.c.) and hydrochloric acid (d. 1·19; 50 c.c.) was treated with potassium thiocyanate (60 g.) and the solution evaporated to dryness in a china-dish on the steam-bath. The residue on crystallisation from boiling water yielded the thiourea derivative in shining thin rhombic plates or leaflets, m.p. 197° (dec.); yield, 85 g. (Found: N, 18·3; $C_7H_9N_3O_2S_2$ requires N, 18·2%.)

2-(Para*sulphonamidobenzeneamino*)-*thiazol* (*III*, $R = R' = H$) or 2-para-*sulphonamidobenzeneiminothiazolin* (*IV*, $R = R' = R'' = H$).—The foregoing thiocarbamide (5·7 g.) and 1 : 2-diochlorether (4 c.c.) in water (20 c.c.) were heated under reflex. After everything had gone into solution, boiling was continued for 45 minutes more. The solution was cooled and made ammoniacal whereby the thiazol derivative was precipitated. It separated from water as a greyish amorphous powder, m.p. about 240° (dec.). Yield, 5·0 g. (Found : N, 16·3; $C_9H_9N_3O_2S_2$ requires N, 16·5%.)

2-Para*sulphonamidobenzeneimino*-3-*allylthiazolin* (*IV*, $R =$ *allyl*, $R' = R'' = H$).—N-*para*sulphonamidophenyl-N′-allylthiocarbamide (5·4 g.) and 1 : 2-dichlorether (2·7 c.c.) in water (30 c.c.) were heated under reflex for 1½ hrs. The clear cooled solution was neutralised with ammonia and the solid that separated on crystallisation from alcohol was obtained in rhombic plates.

m.p. 139·5–41°, yield, 5·8 g. (Found : N, 13·6; $C_{12}H_{13}N_3O_2S_2$ requires N, 14·2%.)

2-Para*sulphonamidobenzeneimino*-3-*phenylthiazolin* (*IV*, *R* = *Ph*, *R'* = *R''* = *H*). —This was prepared as above by heating equimolecular quantities of N-phenyl-N'-*para*sulphonamidophenylthiocarbamide and 1 : 2-dichlorether in water ; m.p. 193°. (Found : N, 12·2; $C_{15}H_{14}N_3O_2S_2$ requires N, 12·7%.)

2-Para*sulphonamidobenzeneimino*-3-*allylthiazolin* (*IV*, *R* = *allyl*, *R'* = *Ph and R''* *H*). —Prepared by heating equimolecular quantities of N'-*para*-sulphonamidophenyl *N'*-allylthiocarbamide and phenacylbromide in water. It crystallised from alcohol in shining prisms or plates, m.p. 209–10°. (Found : N, 11·0; $C_{18}H_{18}N_3O_2S_2$ requires N, 11·3%.)

2-Para*sulphonamidophenylamino*-4-*phenylthiazol* (*III*, *R'* = *Ph*, *R''* = *H*) or 2-para*sulphonamidophenylimino*-4-*phenylthiazolin* (*IV*, *R* = *R''* = *H R'* = *Ph*). — Prepared as above by heating equimolecular quantities of phenacyl bromide and N-*para*sulphonamidophenylthiocarbamide in water or alcohol, m.p. 228 30°. (Found : N, 12·4; $C_{15}H_{14}N_3O_2S_2$ requires N, 12·7%.)

Ethyl 2-para*sulphonamidobenzeneamino*-4-*methylthiazol*-5-*carboxylate* (*III*, *R'* *Me*, *R''* CO_2Et) *or ethyl* 2-para*sulphonamidobenzeneimino*-4-*methyl-thiazolin*-5-*carboxylate* (*IV*, *R* *H*, *R'* = CH_3, *R''* = CO_2Et).—A mixture of ethyl-*α*-bromacetoacetate (17 g. freshly prepared by brominating ethylaceto-acetate with one molecule of bromine at 0° C. according to the method of Conrad and Schmidt[20]), finely powdered N-*para*sulphonamidophenylthio-carbimide (18 g.) and water (200 c.c.) was warmed on the steam-bath with vigorous shaking. The mass gradually turned yellow and shaking was con-tinued till the smell of the ester was not perceptible. After allowing to stand overnight, it was filtered, dissolved in (2N) sodium hydroxide and the clear solution neutralised whereby the thiazol ester separated. It was filtered and on crystallisation from dilute alcohol was obtained as light yellow prisms, m.p. 243 45°, yield, 25 g. (Found : N, 11·9 ; $C_{13}H_{15}N_3O_4S_2$ requires N, 12·3%.)

Ethyl 2-para*sulphonamidobenzeneaminothiazolyl*-4-*acetate* (*III*, *R'* = $-CH\cdot COEt$, *R''* *H*) *or* 2-para*sulphonamidobenzeneiminothiazolinyl*-4-*ethylacetate* (*IV*, *R* *R''* *H*, *R'* = CH_2CO_2Et).—This was prepared as the foregoing by condensing N-*para*sulphonamidopnenylthiocarbamide with an equi-molecular quantity of ethyl-*γ*-bromoacetoacetate (prepared by brominating ethylacetoacetate in carbon disulphide at room temperature[20]). It was obtained as a yellowish brown crystalline powder, m.p. 219–20° (slight dec.). (Found : N, 12·2; $C_{13}H_{15}N_3O_4S_2$ requires N, 12·3%).

Ethyl 2-para*sulphonamidobenzeneamino-4-methylthiazolyl-5-acetate* (*III*, $R' = Me$, $R'' = -CH_2 \cdot CO_2Et$) *or Ethyl* 2-para*sulphonamidobenzeneimino-4-methyl thiazolinyl-5-acetate* (*IV*, $R = H$, $R' = Me$, $R'' = -CH_2 \cdot CO_2Et$).—Finely powdered N-*para*sulphonamidobenzenethiocarbamide (10 g.), ethyl-β-bromolevulinate (12·5 g.) and water (150 c.c.) were warmed on the water-bath with vigorous shaking for about 45 mts. It was then filtered, and the clear filtrate neutralised with ammonia whereby the condensation product separated. It crystallised from alcohol in shining rhombic plates, m.p. 163°, after softening at 154°, yield, 12 g. (Found : N, 11·6; $C_{14}H_{17}N_3O_4S_2$ requires N, 11·8%.)

N-para*sulphonamidophenylpseudothiohydantoin* (*V* or *VI*).—This was prepared by refluxing equimolecular quantities of chloracetic ester or chloracetic acid and N-*para*sulphonamidobenzenethiocarbamide in absolute alcohol for 2 to 3 hours. In the place of the acid or ester, chloracetyl chloride can also be used but the reaction is best conducted in acetone. It crystallised from alcohol in fine needles melting indefinitely between 240–55°. (Found : N, 15·2; $C_9H_9N_3O_3S_2$ requires N, 15·5%.)

When the reaction was carried out in dilute alcohol or water or the refluxing much more prolonged, in addition to the above product, sulphanilamide was also isolated.

2-Para*sulphonamidobenzeneamino-5-iodomethylthiazolin* (*VIII*) *or* 2-para*sulphonamidobenzeneimino-5-iodomethylthiazolidon* (*IX*).—To a solution of N-allyl-N′-*para*sulphonamidophenylthiocarbamide (5·4 g.) in absolute alcohol (50 c.c.) was added iodine (5·1 g.) and refluxed on the steam-bath. The solution was decoloursied in about 15 mts. After boiling for 2 hours more, the solution was diluted and neutralised with ammonia. The oil that separated gradually solidified. From alcohol it separated as a gum and gradually crystallised; m.p. 115–19°, yield, 4·9 g. (Found : N, 10·2; $C_{10}H_{12}N_3O_2S_2I$ requires N, 10·6%.)

4-*Amino*-5-[4′-(2) *thiazolylsulphonamidophenylazo*] *thiouracil* (*X*).—2-N^1-sulphanilamidothiazol (2·5 g.) was diazotised whereby the diazo-compound separated as a yellow mass. The excess of nitrous acid being destroyed with ammonium sulphamate, it was added to a solution of 4-aminothiouracil (1·5 g.) dissolved in sodium hydroxide (20 c.c. of 2·5 N). After stirring for about 15 mts. the dark red solution was acidified with acetic acid whereby the red dye was precipitated. It was filtered, washed well with water and dried; yield, 4 g. (Found : N, 23·4; $C_{13}H_{11}N_7O_3S$ requires N, 24·0%.)

2-(4-N^1-*sulphanilamidobenzenesulphonamido*) *thiazol* (*XI*).—To a solution of 2-N^1-sulphanilamidothiazol (5 g.) in pyridine (6 c.c.) and acetone

(20 c.c.) was added *para*acetaminobenzenesulphochloride (5 g.) and heated on the steam-bath for ½ hour. The solution was diluted with water, filtered and the solid obtained was thoroughly triturated with dilute hydrochloric acid and filtered. The product obtained was boiled with 8 times the quantity of about 3·5 N hydrochloric acid for 45 mts. and filtered. The clear filtrate was neutralised with ammonium hydroxide and the product that separated crystallised from alcohol. Greyish prisms, m.p. 163–4°. (Found: N, 13·6; $C_{15}H_{14}N_4O_4S_3$ requires N, 13·7%.)

9-*Chloroacridine*.—The methods of synthesis of this compound described in literature are lacking in details; the following method gave the compound in good yields and is fairly rapid also.

A solution of equal amounts of 2-chlorobenzoic acid and potassium carbonate was evaporated to dryness first on the steam-bath and then at 110°. The solid mass was finely powdered and then heated under reflux with three times the amount of aniline and a little copper powder for three to four hours. The product was then acidified with dilute hydrochloric acid whereby diphenylaminecarboxylic acid separated. It was filtered, dissolved in dilute sodium hydroxide, filtered and the clear filtrate acidified. The acid that separated was filtered off, washed with water and dried. This was found to be pure enough for the next operation. The foregoing dry acid with 5 to 8 times of phosphorous oxychloride was refluxed first very gently till the vigour of the reaction subsided. After refluxing for two hours it was poured into crushed ice and water, care being taken that during decomposition of the oxychloride the product did not get warmed up. After making it alkaline with ammonia at about 0° to 5°, the chloroacridine which separated and recognisable by the characteristic aromatic odour, was filtered, washed well with ice water and thoroughly dried in a *desiccator*. If in any one of the operations the temperature was allowed to rise or the wet product warmed with any solvent, the chloracridine was converted into acridone showing the blue fluorescence. The above dry chloracridine is pure enough for the subsequent operations and the dry product can be crystallised from absolute alcohol.

Acridine derivatives of formula (XII).—The acridine derivatives mentioned below were prepared by the following general method. One molecule of 9-chloracridine was heated on the steam-bath with 5-8 times the weight of phenol till everything went into solution. Then one molecule of the finely powdered amine ($NH_2 \cdot C_6H_4 \cdot SO_2NH \cdot R$) was added and heated on the steam-bath for two hours more with frequent shaking. The syrupy solution was then diluted with water and made just alkaline with ammonia. The

acridine derivatives separated as gums and soon solidified on scratching. They were all crystallised from alcohol.

N^4-(9′-*acridyl*) *sulphanilamide* (*XII*, $R = H$.)—Orange yellow fine needles, m.p. 245–6°. (Found : N, 12·1; $C_{19}H_{15}N_3O_2S$ requires N, 12·0%.).

2-[N^4-(9′-*acridyl*) *sulphanilamido pyridine*] (*XII*, R = 2-*pyridyl*).—Brown red plates which on powdering became orange yellow, m.p. 268–9° (dec.). (Found : N, 12·6; $C_{24}H_{18}N_4O_2S$ requires N,13·1%).

4-[N^4-(9′-*acridyl*) *sulphanilamido*] *aniline* (*XII*, $R = p$, $C_6H_4 \cdot NH_2$).—Red plates becoming orange yellow on pulverising, m.p. 278–82°. (Found : N, 12·6; $C_{25}H_{20}N_4O_2S$ requires N, 12·7%.)

4-[N^4-(9′-*acridyl*) *sulphanilamido*] *nitrobenzene* (*XII*, $R = p.C_6H_4 \cdot NO_2$).—Orange crystalline powder, not melting below 285°. (Found : N, 11·3; $C_{25}H_{18}N_4O_4S$ requires N, 11·9%.)

4-[N^4-(9′-*acridyl*) *sulphanilamido*] *benzenesulphonamide* (*XII*, R $p \cdot C_6H_4SO_2NH_2$).—Orange prismatic needles, m.p. not below 280°. (Found N, 11·0; $C_{25}H_{20}N_4O_4S_2$ requires N, 11·1%.)

The nitrogen in all these compounds was estimated by the semi-micro-Kjeldahl method of Folin. In the case of the dye and the nitro compound the products were first reduced with sodium bisulphite and then digested.

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Summary

The synthesis of thirteen (N^4) amino-substituted sulphanilamide compounds of thiozal and related derivatives is described. None of them showed any activity in experimental *streptococcal* and *pneumococcal* infections in mice. Five sulphanilamide derivatives of acridine of the above group have also been described. Though some of them possessed considerable activity in the *streptococcal* infections, they were inactive in *pneumococcal* infections. It is apparent that for pronounced antibacterial action the heterocyclic ring should be substituted in the sulphonamide radical leaving a free amino group, which appears to play some significant, but as yet not perfectly understood, rôle in the mechanism of therapeutic action.

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