Pergamon

Bioorganic & Medicinal Chemistry Letters 11 (2001) 137–140

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

New Anti-MRSA Cephalosporins with a Basic Aminopyridine at the C-7 Position

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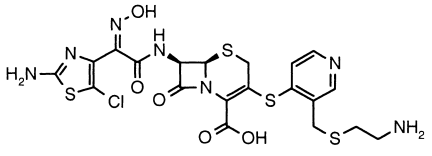
Received 18 September 2000; accepted 25 October 2000

Abstract—Incorporation of a basic aminopyridine into the C-7 position of 3-(amine-substituted arylthio)-3-norcephalosporins, as in **3**, afforded high potency against MRSA and acceptable solubility for intravenous administration.

The usefulness of β-lactam drugs continues to be eroded because of the emergence and spread of resistant pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA).[1] Thus, there have been considerable efforts to restore effectiveness by building potency against such pathogens into this class of antibiotics.[2]

We recently reported the discovery of RWJ-54428 (MC-02,479, **1**), a new cephalosporin that displays potent activity against MRSA.[3] This compound has several unique structural features, notably a 4-pyridinethiol directly linked to the cephalosporin core (i.e., no methylene spacer between them) and a 2-aminoethylthiomethyl substituent. The pyridine ring, in particular, proves to be crucial for both activity and solubility. We[4] and others[5] observed earlier that higher anti-MRSA activity is achieved with more lipophilicity at C-3. The pyridine of **1** is unprotonated at physiological pH and thereby meets this lipophilicity criterion. At acidic pH, on the other hand, the pyridine is positively-charged, thus affording acceptable solubility for intravenous administration.

Compound **2**, with a nonbasic aromatic ring at C-3, displayed excellent antibacterial activity (see Table 1). As expected, however, this compound was sparingly soluble in water due to its zwitterionic nature, which precluded further pharmacological profiling. This lack of solubility also discouraged us from exploring other neutral heteroaromatic ring systems. As a way of attaining the desired level of solubility, we sought to introduce an additional basic functionality at C-7. Since high anti-MRSA activity also appears to correlate with high lipophilicity of the C-7 side chain,[6–8] a similar strategy to that used successfully at C-3 was considered attractive.



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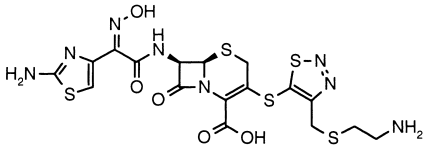
RWJ-54428 (MC-02,479)



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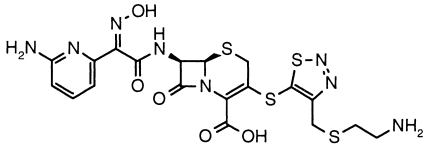

3

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0960-894X/01/$ - see front matter
PII: S0960-894X(00)00603-X

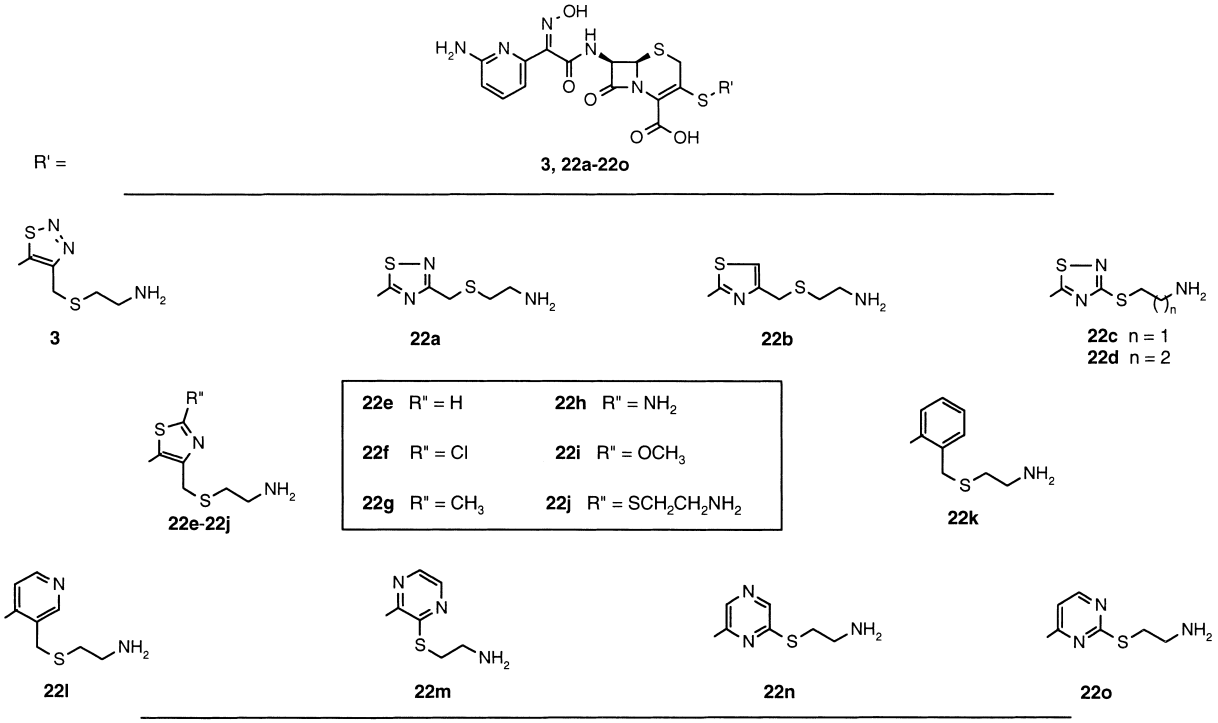
Goto et al. reported that aminopyridines are viable isosteres of the aminothiazole at the C-7 position found in other types of cephalosporins.[9] We envisioned that replacement of the aminothiazole moiety of **2** with the more basic aminopyridine (e.g., as in **3**) would afford enhanced solubility at low pH. Furthermore, the aminopyridine group is expected to be uncharged at physiological pH (predicted p$K_a = \sim 6$), thus providing the required lipophilicity for anti-MRSA activity. We were pleased to discover that **3** displayed excellent anti-MRSA activity (see Table 1) as well as dramatically increased solubility (>20 mg/mL at pH 4.5). Utilizing this new C-7 side chain, we proceeded to synthesize new dibasic analogues with various aromatic systems at C-3 and evaluate their antibacterial activity.[10]

Chemistry

The synthesis of **3** consisted of sequential couplings of three fully protected intermediates: the aminopyridine C-7 side chain **4**, the cephem core **5**,[11] and the thiadiazole thiol **6** (Scheme 1). Reaction of the enol mesylate **5** with the thiol **6** in a biphasic medium (EtOAc/aq sodium bicarbonate) provided **7**. Importantly, no unwanted C-2 double bond isomerization[12] was observed under these conditions. Subsequent acylation of **7** by mixing with the acid **4** and phosphorus oxychloride followed by simultaneous removal of all the protecting groups afforded the desired **3**.[13]

The aminopyridine **4** was synthesized as shown in Scheme 2. Commercially available 2,6-pyridinedicarboxylic acid **9** was converted to the monoamino acid **10** via Curtius rearrangement, and then to the methyl ester **11**. The protected ketoester **14** was obtained by a modified literature method:[9] Pummerer rearrangement of the sulfoxide **12** with trifluoroacetic anhydride followed by addition of methanol gave the dithioorthoformate **13**, which was oxidized with sodium perborate to form the methyl ester **14**. Treatment of **14** with hydroxylamine afforded a mixture of *syn*- and *anti*-oximes (ratio

Table 1. In vitro anti-MRSA activities[a] of dibasic cephalosporins



Compounds	**MRSA**[b] 76	**MRSA** ATCC 335893	**MRSA**[b] Spain #356	Compounds	**MRSA** 76	**MRSA** ATCC 33593	**MRSA** Spain #356
1	1	1	1	**22h**	8	2	4
2	1	1	1	**22i**	4	4	4
3	1	1	1	**22j**	1	1	1
22a	4	2	4	**22k**	1	1	1
22b	2	1	2	**22l**	1	2	2
22c	2	2	2	**22m**	2	2	2
22d	2	2	2	**22n**	16	8	16
22e	2	1	2	**22o**	16	8	16
22f	2	1	1	**Imipenem**	32	32	32
22g	2	2	2	**Vancomycin**	0.5	1	1

[a]MICs (minimum inhibitory concentrations, μg/mL) were determined in Mueller–Hinton broth supplemented with 2% NaCl. MICs were read after 24 h incubation at 35 °C.
[b]Clinical isolates.

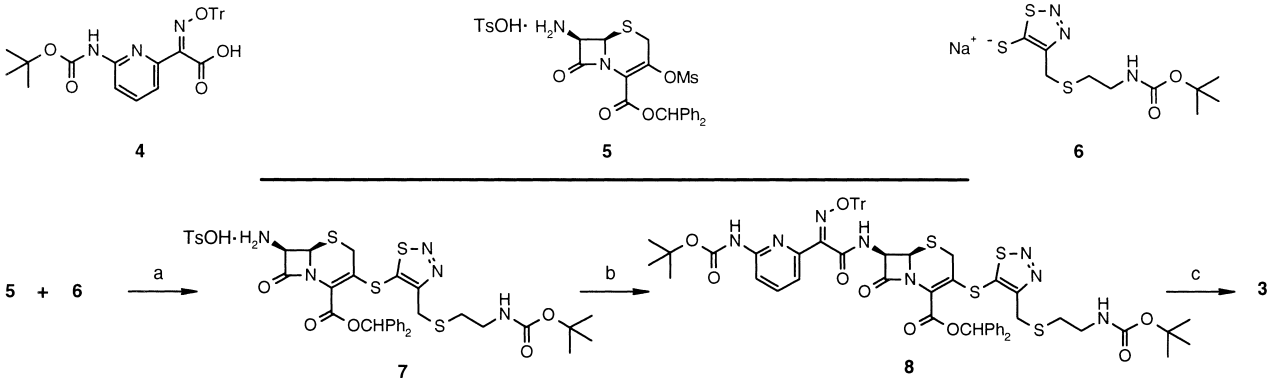
of ca. 5:1), which were separated after tritylation. Hydrolysis of the *syn*-isomer **16** afforded the protected C-7 side-chain acid **4**.

The synthesis of the thiadiazole intermediate **6** is summarized in Scheme 3. Starting with 1,3-dichloroacetone, the appropriately functionalized 1,2,3-thiadiazole **20** was constructed by Hurd–Mori cyclization.[14] Both displacement with 2-aminoethanethiol and subsequent *N*-*t*-BOC protection, giving **21**, were achieved in one pot. Finally, base-promoted β-elimination, with vacuum assisted removal of the volatile acrylic ester by-product, afforded **6** as the sodium salt.

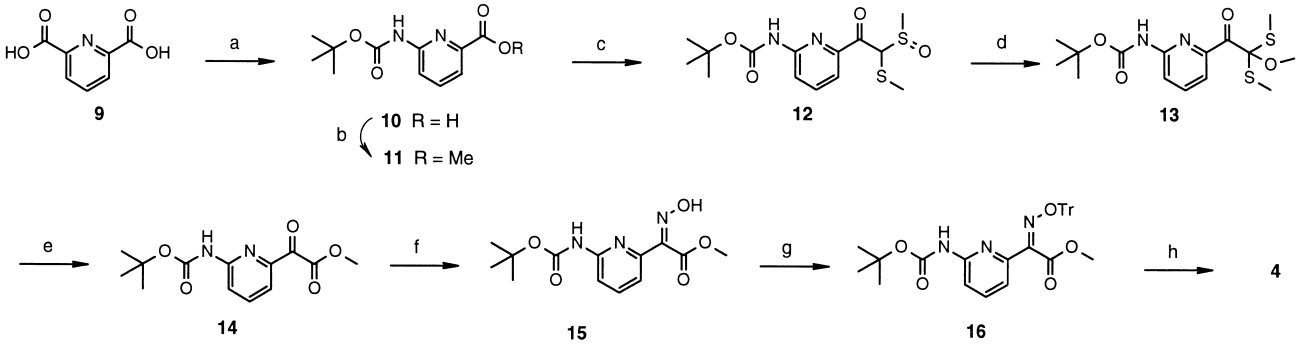
In a similar way, other C-3 aryl analogues **22a**–**22o** were obtained from couplings of **4** and **5** and the corresponding aryl thiols.

Biological Results

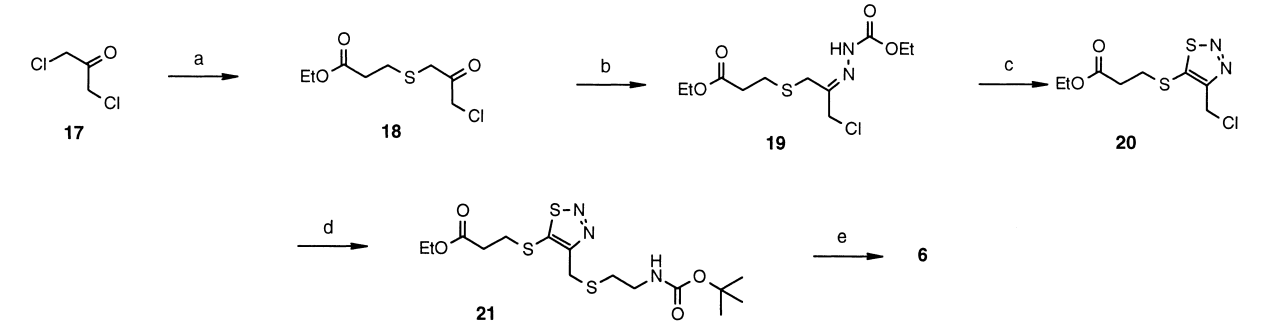
The in vitro antibacterial activity of the newly-prepared cephalosporins was determined against MRSA strains by broth microdilution assay as recommended by the NCCLS.[15] The results are shown in Table 1, with both imipenem and vancomycin included as reference agents.



Scheme 1. Reagents and conditions: (a) EtOAc–aq $NaHCO_3$, 99%; (b) **4**, $POCl_3$, Hunig's base, THF, 45%; (c) TFA, Et_3SiH, CH_2Cl_2, 84%.



Scheme 2. Reagents and conditions: (a) $(PhO)_2PON_3$, TEA, *t*-BuOH, 45%; (b) $POCl_3$, DMF, EtOAc; then MeOH, 98%; (c) NaH, $CH_3SCH_2S(O)CH_3$, DMF, 65%; (d) TFAA, Py, CH_2Cl_2; then MeONa, 85%; (e) $NaBO_3$, AcOH, 40%; (f) NH_2OH, Py, EtOH, 100%; (g) TrCl, TEA; then chromatography, 84%; (h) NaOH, *i*-PrOH–H_2O, 92%.



Scheme 3. Reagents and conditions: (a) $EtO_2CCH_2CH_2SH$, TEA, THF; (b) NH_2NHCO_2Et, cat. TsOH, mol sieve, CH_3CN; (c) $SOCl_2$, CH_2Cl_2, 45% from **17**; (d) NaI, $HSCH_2CH_2NH_2$, *t*-BOC_2O, aq $NaHCO_3$–dioxane, 85%; (e) MeONa, *i*-PrOH, 100%.

Most of compounds tested displayed good to excellent anti-MRSA activity. Activity trends in six-membered ring analogues (**22k** >**22l** >**22m**) reflect the importance of lipophilicity. On the other hand, the five-membered ring thiadiazole **3** was somewhat more potent than the thiazole **22e**, suggesting that other factors such as electronic effects might also be operative. While the aminoethylthiomethyl ring appendage could be positioned either *ortho* (1,2) or *meta* (1,3) to the cephem core in the thiazole series (i.e., **22b** and **22e**), the *meta* analogue **22a** was inferior in activity to the *ortho* analogue **3** in the thiadiazole series. The *meta* analogues **22n** and **22o** in the six-membered ring diazine series were also less active than the *ortho* analogue **22m**. With the exception of **22j** (possessing an extra basic amine), the activities of the series of 1,3-thiazol-5-yl analogues (**22e**–**22i**) appear to correlate with lipophilicity.

Unlike that of zwitterionic compound **2**, solubility of this series of compounds was sufficient to conduct various pharmacological studies. Results of further profiling of selected compounds, including in vivo efficacy, serum effects and pharmacokinetic properties, have been presented.[10]

Conclusions

A new C-7 side chain in which the aminothiazole is replaced with a basic aminopyridine was incorporated into 3-(amine-substituted arylthio)-3-norcephaolsporins. Unlike the zwitterionic compound **2**, the dibasic analogue **3** possessing this C-7 side chain had solubility sufficient for parenteral (iv) administration, while retaining excellent anti-MRSA activity. Various C-3 aromatic ring analogues with this side chain were synthesized and evaluated for their anti-MRSA activity. Some of them were as potent as RWJ-54428, a clinical candidate.

Acknowledgements

The work described herein was conducted as part of a research collaboration with the R.W. Johnson Pharmaceutical Institute. The authors would like to thank Monica Hoang, Cynthia Dinh and Christine Chan for their MIC measurements, and also thank Dr. Ving J. Lee and Dr. Michael N. Dudley for their helpful guidance of this work.

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