Acta Poloniae Pharmaceutica – Drug Research, Vol. 69 No. 4 pp. 623–628, 2012 ISSN 0001-6837
Polish Pharmaceutical Society

DRUG SYNTHESIS

SYNTHESIS OF 2-OXOQUINOLINE-3-CARBOXAMIDE OF AMPICILLIN AND AMOXICILLIN AS INHIBITORS OF PENICILLIN BINDING PROTEIN 1A OF *PSEUDOMONAS AERUGINOSA*

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Abstract: A series of ampicillin and amoxicillin derivatives were prepared by N-acylation with N_1- substituted 1, 2-dihydro-2-oxoquinoline carboxylic acids by Schotten-Baumann procedure and evaluated as inhibitors of *Pseudomonas aeruginosa* using molecular docking study. Most of the derivatives showed remarkable activity against Gram positive and Gram negative bacteria *in vitro*.

Keywords: 2-oxo-quinoline, ampicillin, amoxicillin, molecular docking, antibacterial activity

A second generation penicillins active against *Pseudomonas* species were obtained by acylation of the side chain group of ampicillin and amoxicillin by exploring a variety of heterocyclic acylating agents resulted in the synthesis of new antibiotics (1–4).

A large number of pharmacologically active molecules that have been found for clinical use have been synthesized through the derivatization of quinolones.

Quinolone derivatives have significant biological activities including antibacterial (5, 6), anticonvulsant (7, 8) and antithyroid (9–11). Also amide derivatives of substituted 2-oxo-quinoline acids are showing anticancer (12–14) and antitubercular activity (15–18).

Quinolinoyl derivatives were added to the α-amino group of penicillin and successfully improved their antibacterial activity (19–22). However, there are fewer reports on the synthesis and antibacterial activity of N_1-substituted quinoline-2(1H)-ones-penicillins.

Keeping in mind to improve the activity of parent drugs we have synthesized a series of ampicillin and amoxicillin analogues containing an N_1-substituted 2-oxoquinoline-3-carboxamide of ampicillin and amoxicillin and evaluate their dock score using LigandFit method with PBP1A of *Pseudomonas aeruginosa*.

EXPERIMENTAL

Chemistry

All the melting points were determined in open capillaries using Toshniwal melting point apparatus and were uncorrected. The IR spectra were recorded in the solid state (KBr discs) on a Perkin Elmer Spectrum One FT-IR spectrometer and ^{1}H-NMR spectra in DMSO were recorded on a Bruker 300 MHz instrument. Chemical shifts are reported in parts per million (ppm) using tetramethylsilane (TMS) as an internal standard. All exchangeable protons were confirmed by D_2O exchange method. An elemental analyses (C, H, N) were performed on a Carlo Erba 1108 analyzer and were within ± 0.4% of theoretical values. The mass spectrum was recorded on a Thermo Finnigan ion trap mass spectrometer equipped with an electrospray ionization source. The homogeneity of the compounds was monitored by ascending thin layer chromatography (TLC) on silica gel-G (Merck) coated aluminum plates, visualized by iodine vapor and UV light. The mobile phase was methanol : chloroform (1:9, v/v).

General procedure for the synthesis of compounds 2a-j

Triethylamine (0.015 mol) was added to suspension of 0.01 mol of corresponding 2-oxoquino-

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line-3-carboxlic acid in 20 mL of dry dichloromethane under stirring. The mixture was cooled to –10 – –12°C and 0.015 mol of ethylchloroformate in 10 mL of dry dichloromethane was added dropwise, at constant temperature. Then, reaction mixture was stirred for 1 h at the same temperature. The compounds **2a–j** were used without purification for acylation procedure.

General procedure for the synthesis of compounds 3a–j and 4a–j

To a cooled suspension of 0.01 mol of ampicillin in 10 mL water and 20 mL of acetone at 0°C, 0.015 mol of triethylamine was added dropwise resulting in a clear solution. This solution was cooled to –10 – –12°C and added dropwise to cooled (to –10°C) suspension containing the carbonate of corresponding acid. Stirring was continued for 45 min. at –10°C and then 3 h at room temperature. Organic solvent was removed by distillation under reduced pressure. The resulting residue was transferred in 25 mL of water and 25 mL of ethyl acetate was added. The mixture was cooled and acidified with 6 M HCl to pH 2.5 and extracted twice with ethyl acetate (25 mL portions). The organic layer was separated, washed with brine, dried over anhydrous sodium sulfate and evaporated in vacuum. The final yellowish powder obtained was recrystallized from acetone in every case.

The physico-chemical data of all compounds are shown in Table 1. The spectral data of compound **3a**: IR (KBr, cm^{-1}): 1782 (β-lactam), 1720 (COOH), 1667 (NHCO); ^{1}H-NMR (DMSO-d_6, δ, ppm): 1.39 (s, 3H, 2α-CH_3),1.54 (s, 3H, 2 β-CH_3), 3.73 (s, 3H, N-CH_3), 4.20 (s, 1H, 3-CH), 5.38 (d, 1H, J = 3.9 Hz, 5-CH), 5.52 (dd, 1H, J_1 = 4.2 Hz, J_2 = 7.8 Hz, 6-CH), 5.94 (d, 1H, J =7.8 Hz, 10-CH), 7.25–7.42 (m, 5H, Ar-H), 7.47–8.00 (m, 4H, 5'-H, 6'-H, 7'-H, 8'-H), 8.82 (s, 1H, 4'-H), 9.32 (d, 1H, J =7.8 Hz, 6-NH), 10.76 (d, 1H, J = 7.8 Hz, α-NH); MS: *m/z* (%): 535.07 (100) [M + 1].

The spectral data of other compounds **3b–j** and **4a–j** exhibit analogous patterns.

Ligand-protein interaction

Virtual screening of all the synthesized compounds was carried out by using Accelry's Discovery Studio by LigandFit method. The protein-

Table 1. Physicochemical data of compounds **3a–j** and **4a–j**.

Comp. No.	Yield %	M.p. °C	Empirical formula	Mol. weight	Analysis Calcd. (Found) (%)		
					C	H	N
3a	67	190–191	$C_{27}H_{26}N_4O_6S$	534.58	60.66 (60.83)	4.90 (4.75)	10.48 (10.77)
3b	61	189–190	$C_{28}H_{28}N_4O_6S$	548.61	61.30 (61.51)	5.14 (5.26)	10.21 (10.07)
3c	59	191–193	$C_{28}H_{28}N_4O_6S$	548.61	61.30 (61.17)	5.14 (5.29)	10.21 (10.36)
3d	63	187–188	$C_{28}H_{28}N_4O_7S$	564.61	59.56 (59.29)	5.00 (4.85)	9.92 (9.71)
3e	60	184–185	$C_{28}H_{28}N_4O_7S$	564.61	59.56 (59.35)	5.00 (4.91)	9.92 (9.66)
3f	68	184–186	$C_{28}H_{28}N_4O_6S$	548.61	61.30 (61.68)	5.14 (5.37)	10.21 (10.09)
3g	59	195–196	$C_{29}H_{30}N_4O_6S$	562.64	61.91 (61.70)	5.37 (5.50)	9.96 (9.80)
3h	60	188–189	$C_{29}H_{30}N_4O_6S$	562.64	61.91 (61.66)	5.37 (5.22)	9.96 (9.77)
3i	58	192–193	$C_{29}H_{30}N_4O_7S$	578.64	60.20 (60.44)	5.23 (5.09)	9.68 (9.51)
3j	65	189–190	$C_{29}H_{30}N_4O_7S$	578.64	60.20 (60.09)	5.23 (5.37)	9.68 (9.43)
4a	66	183–185	$C_{27}H_{26}N_4O_7S$	550.58	58.90 (58.67)	4.76 (4.88)	10.18 (10.38)
4b	58	185–187	$C_{28}H_{28}N_4O_7S$	564.61	59.56 (59.74)	5.00 (5.20)	9.92 (9.70)
4c	63	190–192	$C_{28}H_{28}N_4O_7S$	564.61	59.56 (59.32)	5.00 (5.24)	9.92 (9.66)
4d	64	186–188	$C_{28}H_{28}N_4O_8S$	580.61	57.92 (57.77)	4.86 (4.93)	9.65 (9.37)
4e	59	183–185	$C_{28}H_{28}N_4O_8S$	580.61	57.92 (57.64)	4.86 (4.72)	9.65 (9.81)
4f	61	188–190	$C_{28}H_{28}N_4O_7S$	564.61	59.56 (59.70)	5.00 (4.86)	9.92 (10.03)
4g	55	191–192	$C_{29}H_{30}N_4O_7S$	578.64	60.20 (60.36)	5.23 (5.39)	9.68 (9.83)
4h	60	183–185	$C_{29}H_{30}N_4O_7S$	578.64	60.20 (60.02)	5.23 (5.41)	9.68 (9.49)
4i	54	191–192	$C_{29}H_{30}N_4O_8S$	594.64	58.58 (58.44)	5.09 (5.25)	9.42 (9.68)
4j	57	187–189	$C_{29}H_{30}N_4O_8S$	594.64	58.58 (58.39)	5.09 (5.19)	9.42 (9.59)

ligand interaction were evaluated against Gram negative bacteria *Pseudomonas aeruginosa* at PBP 1A.

Pharmacological activity

All the synthesized derivatives were screened for their antibacterial activity *in vitro* against two Gram positive organisms viz. *Bacillus subtilis* (ATCC No. 11774), *Staphylococcus aureus* (ATCC No. 6538P) and four Gram negative organisms viz. *Pseudomonas aeruginosa* (ATCC No. 27853), *Escherichia coli* (ATCC No. 25922), *Proteus vulgaris* (ATCC No. 13315) and *Salmonella typhimurium* (ATCC No. 23564). Serial dilution method was used for screening the antibacterial tests using nutrient broth media. The solutions were prepared in dimethyl sulfoxide and concentrations were ranging from 1.0 to 15 µg/mL. Negative control was kept to study the effect of dimethyl sulfoxide (DMSO). Ampicillin was used as positive reference standard. The test tubes were incubated for 24 h at 37°C and the MIC was determined by visual turbidity observation of microorganism growth. Assays were performed in triplicate. On the basis of observed MIC's it is concluded that all the prepared derivatives have significant antibacterial activity against Gram positive and Gram negative bacterial strains.

RESULTS AND DISCUSSION

Formation of N-acyl derivatives of ampicillin and amoxicillin **3a–j** and **4a–j** were confirmed on the basis of IR, ^{1}H-NMR, MS data and elemental analyses. The IR spectra of compound (**3a**) did not showed two stretching vibrations for the N-H bond of the primary amino group in the range 3400–3465 cm^{-1} and it contained only one band at 3268 cm^{-1} due to amide NH group and it also showed new peak at 1667 cm^{-1} due to amide carbonyl bond. The ^{1}H-NMR spectrum of **3a** showed doublet at δ 10.76 (J = 7.8 Hz,) due to amide bond on α-NH and this was D_2O exchangeable. It showed two multiplets resonating at δ 7.25–7.42 ppm and 7.47–8.00 ppm due to aromatic ring of ampicillin and 2-oxoquinoline ring protons, respectively, and a singlet was also observed at δ 8.82 ppm for proton 4'-H of 2-oxoquinoline. Further evidence for the formation of compound **3a** was confirmed by mass spectra. The mass spectrum of compound **3a** showed a molecular ion peak at m/z 535.07, which was in conformity with the molecular formula $C_{27}H_{26}N_4O_6S$. The elemental data found for the formula $C_{27}H_{26}N_4O_6S$ agreed with calculated as given in Table 1.

Table 2. Summary of docking information of the top ranked poses in each compound **(3a–j)** and **(4a–j)**

Compd.	LigScore1	LigScore2	-PLP1	-PLP2	JAIN	-PMF	Dockscore
3a	3.24	2.13	83.09	82.5	4.29	94.17	53.842
3b	2.64	4.73	83.35	82.68	2.96	118.35	59.144
3c	4.54	6.28	74.85	66.88	-1.22	49.1	66.919
3d	4.64	4.67	96.28	91.72	5.75	102.94	48.846
3e	3.43	5.09	84.16	83.11	3.34	105.49	63.413
3f	3.6	4.85	88.82	84.6	2.32	128.66	49.831
3g	3.62	4.26	84.48	87.23	3.31	104.77	51.131
3h	5.0	4.61	88.67	85.35	6.08	71.94	52.668
3i	2.93	4.86	97.43	81.54	0.46	60.94	52.11
3j	4.92	5.21	99.45	99.41	4.57	107.44	52.678
4a	2.49	3.96	85.02	91.26	4.26	107.28	53.479
4b	5.05	4.78	104.99	103.19	4.58	74.55	54.943
4c	5.33	5.66	109.16	101.84	4.16	104.75	60.401
4d	4.03	4.03	86.14	79.28	1.2	81.6	53.164
4e	3.11	4.16	79.31	74.67	0.75	113.42	46.007
4f	4.24	4.19	10.16	100.24	4.75	90.88	47.682
4g	2.52	4.56	74.67	80.48	2.48	102.98	48.219
4h	5.18	5.47	104.45	95.54	2.35	49.04	56.791
4i	2.66	4.26	79.75	84.74	1.72	95.93	43.692
4j	2.51	4.23	72.84	75.44	3.54	111.37	54.46

Table 3. Results of test for antibacterial activity in MIC's (μg/mL)

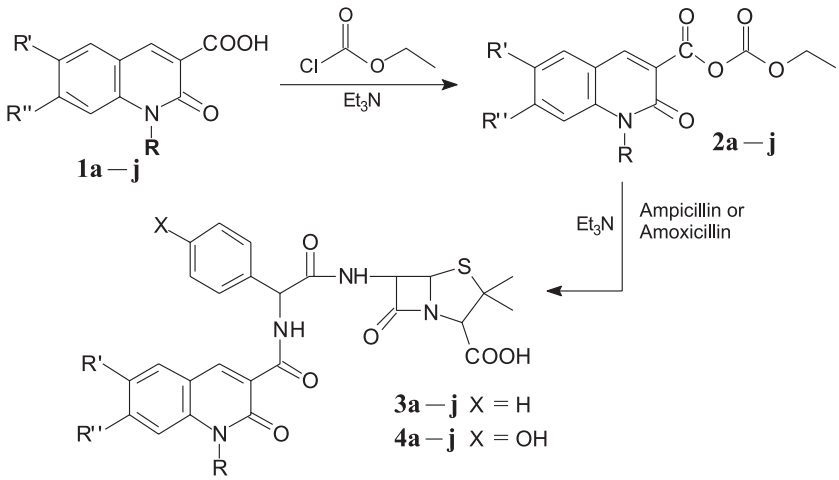
Compd.	*B. subtilis*	*S. aureus*	*E. coli*	*P. aeruginosa*	*P. vulgaris*	*S. typhimurium*
3a	4.5	5.0	5.0	2.5	5.5	7.0
3b	5.0	6.5	7.0	3.5	9.5	10
3c	5.0	6.5	8.0	3.5	9.0	11
3d	3.5	4.5	7.0	3.0	6.5	8.0
3e	4.0	5.0	8.0	3.5	8.5	8.0
3f	5.0	5.0	5.5	3.5	6.5	7.5
3g	6.5	7.5	9.0	4.0	10	12
3h	6.5	7.0	9.5	5.0	10	12.5
3i	6.0	8.0	8.0	4.5	9.0	9.0
3j	6.0	7.5	8.5	5.0	5.5	9.0
4a	4.0	5.5	7.0	3.0	7.0	8.5
4b	5.5	7.0	9.5	6.0	10.5	11.5
4c	5.0	7.0	9.5	4.5	11	11
4d	4.0	8.0	8.5	5.0	10	10
4e	4.0	7.5	8.5	4.0	9.0	9.0
4f	5.5	6.5	9.0	3.5	8.5	9.5
4g	7.0	10	10	5.5	10.5	12.5
4h	7.0	10	10	6.5	11	11
4i	6.5	9.0	9.0	6.5	9.5	10.5
4j	6.5	9.0	8.5	5.5	11.5	10
AMPI	6.5	7.5	5.0	–	2.5	2.0

AMPI = ampicilin

All synthesized compounds were studied for binding affinities towards the protein. Compounds **3a–j** and **4a–j** showed binding affinity with the PBP1A of *Pseudomonas aeruginosa*. As a result of docking, 10 different conformations were generated for all synthesized compounds but only top ranked docked complex scores were copied from the table browser view of discovery studio binding affinity analysis and its all scoring results are given in Table 2.

The score values includes Ligscore 1 and 2 (Protein Ligand Affinity Energy), PLP 1, PLP 2 (Steric and H-bonding intermolecular function), JAIN (Sum of five interaction terms namely: lipophilic interactions, polar attractive interaction, polar repulsive interactions, solvation of the protein and ligand, an entropy term for the ligand), PMF (developed based on statistical analysis of the 3D structures of protein-ligand complexes, scores are calculated by summing pairwise interaction terms over all interatomic pairs of the receptor-ligand complex; a higher score indicates a stronger receptor-ligand binding affinity) and DockScore (candidate ligand poses are evaluated and prioritized according to the DockScore functions). The determination of the ligand binding affinity was calculated using the shape-based interaction energies of the ligand with the protein. Larger score values indicate better ligand-binding affinity. Compound **3c** and **3e** showed the highest dock score.

The investigation of antibacterial screening revealed that all the tested compounds showed considerable and varied activity against the Gram positive and Gram negative bacteria. Compound **3d** showed maximum activity against Gram positive bacteria even less MIC than parent drug and **3a** showed the same against Gram negative bacteria. It has been observed that N'-methyl substituted derivatives showed better activity than the ethyl substituted derivatives. Also, the ampicillin derivatives showed better activity than the amoxicillin derivatives. Compounds **3a** and **3i** showed maximum activity against *Pseudomonas aeruginosa*, since the reference compound was not active against this strain. The results of antibacterial screening are presented in Table 3.



	a	b	c	d	e	f	g	h	i	j
R	CH_3	CH_3	CH_3	CH_3	CH_3	C_2H_5	C_2H_5	C_2H_5	C_2H_5	C_2H_5
R'	H	CH_3	H	OCH_3	H	H	CH_3	H	OCH_3	H
R''	H	H	CH_3	H	OCH_3	H	H	CH_3	H	OCH_3

Scheme 1. The synthetic protocols of the 2-oxo-quinoline-3-carboxamide of ampicillin and amoxicillin

CONCLUSION

We have synthesized twenty N-acyl derivatives of ampicillin and amoxicillin using mixed anhydride method with moderate yield. All derivatives are good inhibitors of PBP1A of *Pseudomonas aeruginosa*. The broad spectrum of antibacterial activity was observed for all tested compounds. It was observed that all compounds are excellent *anti Pseudomonas* as compared to standard drug used.

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Received: 17. 03. 2011