Indian Journal of Chemistry
Vol. 62, January 2023, pp. 72-75
DOI: 10.56042/ijc.v62i1.70391

Investigation of 2-oxopyrrolidine 5-carboxylic acid amides derivatives as potential anti-tubercular agents based on the similarity screening results from molecular fingerprints and SWISS SIMILARITY

K P Nagasree, W A Umarani, K P Sony K, K S Kumar & M Murali Krishna Kumar*

Pharmaceutical Chemistry Research Lab, AU College of Pharmaceutical Sciences, Andhra University, Visakhapatnam – 530 003, India

*E-mail: umaraniwunnava@gmail.com

Received 23 April 2021; accepted (*revised*) *18 November 2022*

Many open source *in silico* tools are available for drug discovery. This paper attempts to correlate the results obtained from two such tools with results obtained from *in vitro* screening. Discovery of novel Anti-TB agents became a necessity due to the ever increasing problem of drug resistance. As heterocyclic compounds are known to possess potent biological activity, here an attempt has been made to synthesize pyrazolidine derivatives from pyroglutamic acid. Further the synthesized derivatives are analyzed by IR, NMR and Mass spectral studies and screened for anti-tubercular and antimicrobial activity.

Keywords: Pyroglutamic acid, 2-oxopyrrolidine 5-carboxylic acid amides derivatives, anti-tubercular activity, CHEMDES, SWISS SIMILARITY

Mycobacterial cell walls contain poly-L-glutamate or glutamine as an integral component. Pyroglutamic acid residues are found in peptides at the N-terminal end[1,2]. Antimetabolites have been explored as therapy against various disease conditions. The same strategy has been explored in our design of amides of pyroglutamic acid as potential anti tubercular agents. Synthesis and screening are reported in this research article. The statistical data periodically released by the World Health Organization reflects the need for continuous research into novel and safer anti tubercular therapy; though effective medication in the form of first line anti-TB drugs (INH) and second line drugs are available[3,4,5]. Based on the above literature a SWISS SIMILARITY search by ShapeIT[6] screening method was done with 2-oxopyrrolidine 5-carboxylic acid amide as the query molecule.

Experimental Details

All the chemicals were purchased from Aldrich Chemical Company (USA) and were used without further purification. The reactions were monitored by pre-coated aluminum silica gel TLC plates. Iodine vapors are used as visualizing agents. Melting points (m.p.) were determined using an SRS-EZMelt automated melting point instrument, without correction. The IR spectra were recorded on BRUKER FT-IR (software - OPUS 6.4) spectrometer using KBr disc method and the values were expressed in cm^{-1}. The ^{1}H-NMR spectra of the compounds were recorded in DMSO-d6 or $CDCl_3$ with BRUKER AVANCE 400 MHz NMR spectrometer (software – Topspin 3.5) and chemical shifts were expressed in δ (ppm). Shifts reported are relative to the signal of the solvent used in each case and coupling constants are reported in Hz (s: singlet, bs: broad singlet, d: doublet, t: triplet, dd: double doublet, dt: double triplet, m: multiplet).

Characterization of synthesized compounds

5-Oxo-N-phenyl pyrrolidine-2-carboxamide (KSK 09): Yield 36.253%; m.p. 117°C; Anal. Calc for $C_{11}H_{12}N_2O_2$; C, 64.69; H, 5.92; N, 13.72; O, 15.62; found: C, 64.67; H, 5.90; N, 13.71; O, 15.59; IR (KBr) υ max: cm^{-1}: 1557.22 (C-H aromatic), 1695 (C=O), 1750 (lactam amide), 3328.07 (lactam N-H); ^{1}H NMR (DMSO, 400 MHz) δ: 2.01,2.355 (m ,2H, CH_2), 2.1-2.3 (m, 2H, CH_2), 4.21 (q, 1H, CH, J=3.56 Hz), 7.09 (t, 1H, CH, J=14.8 Hz), 7.32 (t, 2H, Ar-CH J=15.6 Hz), 7.63 (d, 2H, Ar-CH, J=7.6 Hz),7.894 (s,1H, NH), 10.03 (s, 1H, NH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 25.79(CH_2), 29.71(CH_2), 56.85(CH), 119.81(Ar-C), 123.99(Ar-C), 129.24(Ar-C), 139.26 (Ar-C), 171.26(C=O), 177(C=O).

5-(4-Phenylpiperazine-1-carbonyl)pyrrolidine-2-one (KSK 10): Yield 43.08%, m.p. 191°C; Anal. Calc for $C_{15}H_{19}N_3O_2$; C, 65.91; H, 7.01; N, 15.37; O,11.71; found C,65.93; H,7.00; N, 15.35; O, 11.69; IR (KBr) υ max: cm^{-1}:1607.22 (C-H aromatic), 1795 (C=O), 1950 (lactam amide), 3328.07 (lactam N-H); ^{1}H NMR (DMSO, 400 MHz) δ: 2.12, 2.388(m, 2H, CH_2), 2.35(m, 2H, CH_2), 3.15(t, 2H, $2CH_2$, $6CH_2$, J=9.2Hz), 3.71(dd, 2H, $3CH_2$, $5CH_2$, J=5.2Hz), 5.0(q, 1H, CH, J=3.6 Hz), 6.84(t,1 H, Ar-4CH, J= 14.4Hz), 6.98(d, 1H, Ar-2CH, 6CH, J= 7.6Hz), 7.25(t, 1H, Ar-3CH, 5CH, J= 16 Hz), 7.9(s, 1H, NH); ^{13}C NMR (DMSO, 100 MHz) δ: 28.88(CH_2), 31.95(CH_2), 42.08(CH_2), 49.11(CH_2), 116.47(Ar-2CH, 6CH), 119.98(Ar-4CH), 129.49(Ar-3CH, 5CH), 169.76(C=O), 177.91(C=O).

N-(2,4-diflorophenyl)-5-oxopyrrolidine-2-carboxamide (KSK 11): Yield 56.98%; m.p 191°C; Anal. Calc for $C_{11}H_{10}F_2N_2O_2$; C, 55.00; H, 4.20; F, 1.82; N, 11.66; O, 13.32; found C, 55.01; H, 4.18; F, 1.80; N, 11.65; O, 13.30; IR (KBr) υ max: cm^{-1}:846.95 (C-H aromatic), 1673.05 (C=O), 3276.09 (N-H lactam), 3508.42 (N-H amide); ^{1}H NMR (DMSO, 400 MHz) δ: 1.99, 2.510(m, 1H, CH_2), 2.162-2.33(m,2H,CH_2), 4.26(q,1H,CH, J=0.4 Hz), 7.08(d,1H,Ar-CH), 7.35(dd, 1H, Ar-5CH), 7.803(m, 1H, Ar-3CH), 7.901(s, 1H, NH), 9.855(s, 1H, NH); ^{13}C NMR (DMSO, 100 MHz) δ: 25.90(CH_2), 29.61(CH_2), 56.27(CH), 104.68(Ar-CH), 111.3(Ar-CH), 117.4(Ar-CH), 126.56(Ar-CH), 160.1(Ar-CH), 164.6(Ar-C), 172.35(C=O), 177.96(C=O).

N-(4-bromophenyl)-5-oxopyrrolidine-2-carboxamid*e* (**KSK 12**): Yield 24.64%; m.p 147°C; Anal. Calc for $C_{11}H_{11}BrN_2O_2$; C, 46.66; H, 3.92; Br, 28.22; N, 9.89; O, 11.30; found: C, 46.64; H, 3.90; Br, 28.21; N, 9.86; O, 11.28; IR (KBr) υ max: cm^{-1}: 666.06(C-H aromatic), 1673.05(C=O), 3105.98(N-H lactam), 3309.69(N-H amide); ^{1}H NMR (DMSO, 400 MHz) δ: 1.998, 2.506 (m,2H,CH_2), 2.157 (m,2H,CH_2), 4.164 (q, 1H,CH, J= 12.8 Hz), 7.504 (d,1H,Ar-3CH,5CH,J=2Hz), 7.613 (d, 1H, Ar-2CH, 6CH, J= 2 Hz), 7.883 (s, 1H, NH), 10.182 (s, 1H, NH); ^{13}C NMR (DMSO, 100 MHz) δ: 25.7(CH_2), 30.6(CH_2), 45.1(CH), 118.7(Ar-4C), 123.8(Ar-2C,6C), 131.9(Ar-3C,5C), 137.5(Ar-1C), 172.0(C=O), 177.8(C=O).

5-(4-Benzylpiperazine-1-carbonyl)pyrrolidine-2-one (KSK 14): Yield 15.279%; m.p 190°C; Anal. Calc for $C_{16}H_{21}N_3O_2$; C, 66.88; H, 7.37; N, 14.62; O, 11.14; found; C, 66.84; H, 7.35; N, 14.60; O, 11.15; IR (KBr) υ max: cm^{-1}: 1607.22 (C-H aromatic), 1795(C=O), 1950 (lactam amide), 2863.9 (CH2) , 3328.07 (lactam N-H); ^{1}H NMR (DMSO, 400 MHz) δ: 2.18,2.28(m,2H,CH_2), 2.46(m,2H,CH_2), 2.62 (m,2H,$2CH_2$, $6CH_2$), 3.30(t, 2H, CH_2), 3.62(s, 2H, CH_2), 4.34(t, 1H, CH), 7.06(d,1H, Ar-CH), 7.07 (t, 1H, Ar-CH), 7.14(t, 1H, Ar-CH), 9.56 (s, 1H, NH); ^{13}C NMR (DMSO, 100 MHz) δ: 26.0 (CH_2),31.6(CH_2), 43.3(CH), 47.5(CH_2), 52.1(CH_2), 60.1(CH_2), 127.3 (Ar-4C), 128.5(Ar-3C,5C), 128.9(Ar-2C,6C), 169.1(C=O), 177.9(C=O);

N-(4-chlorophenyl)-5-oxopyrrolidine-2-carboxamide (**KSK 15**): Yield 28.998%; m.p 184°C; Anal. Calc for $C_{12}H_{12}ClN_2O_2$; C, 55.36; H, 4.65; Cl, 14.85; N, 11.74; O, 13.41; found: C, 55.33; H, 4.62; Cl, 14.84; N, 11.72; O, 13.40; IR (KBr) υ max: cm^{-1}: 647.04(C-Cl aromatic), 1500(C-H aromatic), 1667.41(C=O), 3302.47(N-H amide); ^{1}H NMR (DMSO, 400 MHz) δ: 2.178, 2.353(m, 2H, CH_2), 2.203-2.3(m, 2H, CH_2), 4.168(q, 1H, CH, J= 0.4 Hz), 7.386(d, 1H, Ar-2CH,J= 8.8Hz), 7.653(d, 1H, Ar-CH, J= 9.2Hz), 7.886(s, 1H, NH), 10.184(s, 1H, NH); ^{13}C NMR (DMSO, 100 MHz) δ: 25.74(CH_2), 29.56(CH_2), 56.88(CH), 121.39(Ar-CH), 27.56(Ar-CH), 129.16(Ar-CH), 138.22(Ar-CH), 171.95(C=O), 177.94(C=O).

5-(2-(3-Bromo-2-fluorophenyl) acetyl) pyrrolidin-2-one *(*KSK 18): Yield 18.696%; m.p 158°C; Anal. Calc for $C_{12}H_{11}BrFNO_2$; C, 48.02; H, 3.69; Br, 26.62; F, 6.33; N, 4.67; O, 10.66; found: C, 48.01; H, 3.67; Br, 26.60; F, 6.32; N, 4.65; O, 10.64; ^{1}H NMR (DMSO, 400 MHz) δ: 2.003,2.518(m,2H, CH_2), 2.19(m, 2H, CH_2), 4.17(q,1H,CH, J= 3.6 Hz), 7.1(t,1H,Ar-CH,J=6.8 Hz), 7.32(m,1H,Ar-CH), 7.4(d, 1H, Ar-6=CH,J=8.8 Hz), 7.9(s, 1H, NH), 10.32(s, 1H, NH); ^{13}C NMR (DMSO, 100 MHz) δ: 23.6(CH_2), 32.1(CH_2), 45.1(CH),122.2(Ar-C), 124.0(Ar-C),126.8(Ar-C), 128.9(Ar-C), 130.3(Ar-C), 157.3(Ar-C), 172.0(C=O), 177.8(C=O).

N-(4-methylphenyl)-5-oxopyrrolidine-2-carboxamide (**KSK 19**): Yield 49.230%; m.p 210; Anal. Calc for $C_{12}H_{14}N_2O_2$; C, 66.04; H, 6.47; N, 12.84; O, 14.66; found: C, 66.03; H, 6.45; N, 12.82; O, 14.64; IR (KBr) υ max: cm^{-1}: 669.49(aromatic CH_3), 1552(C-C aromatic), 1662(C=O), 3124(N-H lactam), 3326.65(N-H amide); ^{1}H NMR (DMSO, 400 MHz) δ: 1.98, 2.21(m, 2H, CH_3), 2.15(m, 1H, CH_2), 2.357(m, 1H, CH), 4.173(q, 1H, CH, j= 0.4 Hz), 7.124(d, 1H, Ar-3CH, 5CH,J=8 Hz), 7.507(d, 1H, Ar-2CH, 6CH,J=8 Hz), 7.884(s, 1H, NH), 9.960(s, 1H, NH); ^{13}C NMR (DMSO, 100 MHz) δ: 25.7(CH_2),

31.6(CH_2), 24.3(CH_3),45.1(CH), 121.5(Ar-2C,6C), 129.3(Ar-3C,5C), 134.0(Ar-4C), 135.5(Ar-1C), 172.0(C=O), 177.8(C=O).

N-(2-aminophenyl)-5-oxopyrolidine-2-carboxamide (**KSK 20**): Yield 23.56%; m.p 183; Anal. Calc for $C_{11}H_{13}N_3O_2$; C, 60.23; H, 5.98; N, 19.17; O, 14.60; found: C, 66.20; H, 5.95; N, 19.15; O, 14.58; ^{1}H NMR (DMSO, 400 MHz) δ: 2.18, 2.58(m,2H,CH_2), 2.34(m,2H,CH_2), 4.334(q,1H,CH), 6.45(m,1H,, Ar-CH), 6.58(t,1H, Ar-CH), 6.78(t, 1H, CH), 7.402(d, 1H, Ar-CH), 7.94(s, 1H,NH), 9.80(s, 1H, NH); ^{13}C NMR (DMSO, 100 MHz) δ: 25.7(CH_2), 3.16(CH_2), 45.1(C), 116.5(Ar-3C), 119.0(Ar-5C), 122.4(Ar-6C), 125.2(Ar-4C), 125.5(Ar-1C), 172.0(C=O), 177.8(C=O).

N-(3,4-diflorophenyl)-5-oxopyrrolidine-2-carboxamide (**KSK 22**): Yield 32.129%; m.p 194; Anal. Calc for $C_{11}H_{10}F_2N_2O_2$; C, 55.00; H, 4.20; F, 15.82; N, 11.66; O, 13.32; found C, 55.00; H, 4.18; F, 15.80; N, 11.64; O, 13.31; IR (KBr) υ max: cm^{-1}: 1086.37(C-F aromatic), 1430.58(C-H aromatic), 1879.04(C=O), 3084.24(N-H lactum), 3342.63(N-H amide); ^{1}H NMR (DMSO, 400 MHz) δ: 2.178, 2.515(m, 2H, CH_2), 2.35(m, 2H, CH_2), 4.173(q, 1H, CH), 7.366(d ,1H, Ar-2CH, J=12.4Hz), 7.432(d, 1H, Ar-3CH, J= 9.2Hz), 7.813(m, 1H, Ar-6CH), 7.925(s, 1H, NH), 10.320(s, 1H, NH); ^{13}C NMR (DMSO, 100 MHz) δ: 25.7(CH_2), 31.6(CH_2), 45.1(CH), 111.7(Ar-2C), 117.3(Ar-C), 118.8(Ar-C), 135.7(Ar-C), 145.1(Ar-C), 149.7(Ar-C), 172.0(C=O), 177.8(C=O).

Results and Discussion

A total of ten oxopyrrolidine-2-carboxamide derivatives were synthesized by using established protocols. The compounds were obtained in good yield and were characterized by using spectral analysis including NMR, IR and Mass. Pyroglutamic acid (1 eqv.) was dissolved in DMF (2-3 mL) and EDC.HCl (1.1 eqv.), HOBT (1.1 eq.) were added slowly to the solution under stirring conditions. Then after 10 min, aniline (1 eqv.) was added dropwise to the reaction. To the above solution N-ethyl diisopropylamine (0.6 eqv.) was added and stirred overnight. The reaction was monitored by TLC. After the completion of reaction, water was added slowly with stirring and the reaction mixture was extracted with ethyl acetate. Ethyl acetate layer was distilled under reduced pressure and purification of compound was done by column chromatography. The synthesis scheme is shown in Scheme 1. The synthesized compounds were screened for antimicrobial and anti-tubercular activities.

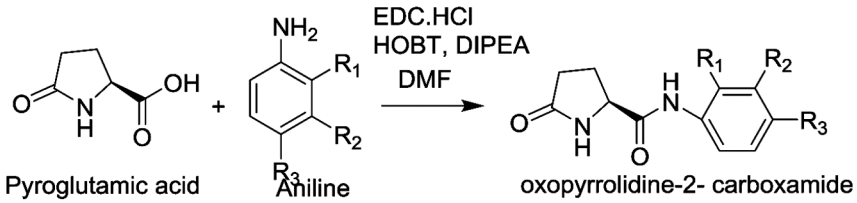
A SWISS SIMILARITY search by ShapeIT[6] screening method was done with 2-oxopyrrolidine 5-carboxylic acid amide as the query molecule. and it was found to have similarity score of 0.8 with ethambutol. Fingerprint based similarity search was done between ethambutol and 2-oxopyrrolidine 5-carboxylic acid amide; by using Chemdes. Different types of fingerprint descriptors like Daylight type, MAACS, Morgan, Atom pairs and Topological were calculated with different scoring measures as shown in Table 1. A good cosine correlation score between the two molecules was observed with Daylight type and Topological fingerprint descriptors. The score obtained from these descriptors was that nearest to the ShapeIT score.

In vitro antimicrobial screening

The antimicrobial susceptibility testing was performed *in vitro* by agar well diffusion method[7,8]. The results were expressed by measuring the zone of inhibition. The zone of inhibition of the synthesized compounds was compared with standard drugs Rifampicin and Ketoconazole for antibacterial and antifungal activity, respectively. The test organisms used were: Gram-positive bacteria - *Staphylococcus aureus,* Gram-negative bacteria- *Escherichia coli* and fungi- *Candida albicans.* None of the compounds had shown antimicrobial activity comparable to that of the standard.

In vitro anti-tubercular activity screening – MABA assay

The test compounds were screened for preliminary anti-TB activity against pathogenic strains of M. *tuberculosis* $H_{37}Rv$ (ATCC 27294), using standard procedure reported for Micro plate



Scheme 1 — Synthesis of oxopyrrolidine-2-carboxamide

Table 1 — Fingerprint similarity scores from CHEMDES

Fingerprint type	Tanimoto score	Dice score	Cosine score
Daylight type	0.40	0.57	0.6
MAACS	0.36	0.53	0.53
Atom Pairs	0.08	0.15	0.18
Morgan	0.016	0.03	0.04
Topolgical	0.40	0.57	0.6

Table 2 — Anti-tubercular activity of the synthesized compounds

Compound code	MIC (µg/mL)
KSK 09	25
KSK 10	25
KSK 11	25
KSK 12	6.25
KSK 14	6.25
KSK 15	12.5
KSK 18	3.12
KSK 19	1.6
KSK 20	12.5.
KSK 22	6.25

Alamar Blue assay (MABA)[9,10,11] without any modifications. The anti-tubercular activity results were presented in Table 2.

Among 10 compounds, 4-methyl phenyl oxapyrrolidine-2-carboxmide had shown good anti-TB activity with an MIC 1.6 µg/mL. 3-bromo-2-floro phenyl oxapyrrolidine-2-carboxmide had shown anti-TB activity with an MIC 3.12 µg/mL. 4-bromo phenyl, difluoro phenyl and phenyl piperizine substituted oxapyrrolidine-2-carboxamide had shown potent anti-TB activity with an MIC 6.25 µg/mL. This observation specifies that presence of electron withdrawing element has significant effect the anti-tubercular activity.

Conclusion

Among the ten compounds synthesized KSK 19 showed potent anti TB activity against MtbH37Rv with an MIC 1.6 µg/mL. Further KSK 12, 14, 18, 22 compounds showed activity at MIC 6.25 µg/mL. These results prove that the results from SWISS SIMILARITY search and the fingerprint descriptor scores obtained from CHEMDES are reliable sources for virtual similarity screening of molecules.

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