Synthesis and antibacterial evaluation of Valproic Acid Amides

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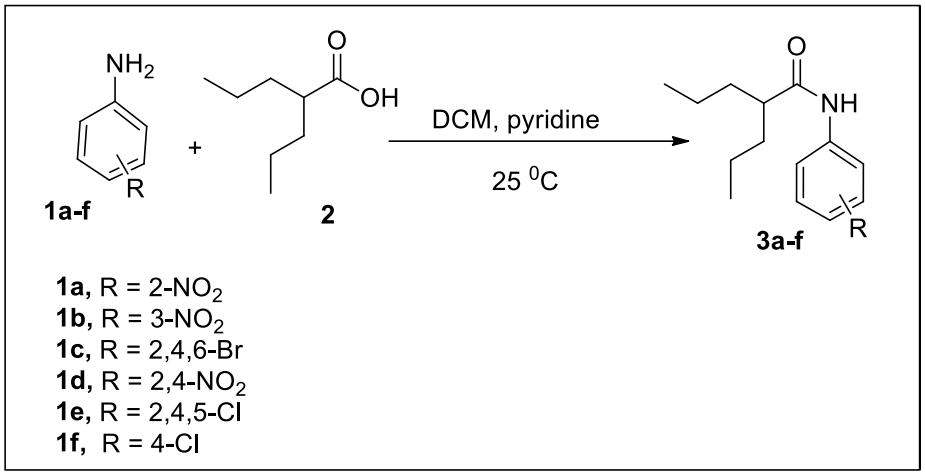
Abstract: VPA has potential therapeutic applications in other central nervous system (CNS) disorders and in various cancer types. Valproic acid also showed potent biological activities the antibacterial, antimicrobial, and anti-inflammatory. Various methodologies represented for the synthesis of nitrogen based valproic acid derivatives. In the present work aniline substituted valproic acid derivatives synthesized (3a-f) in the 85% to 91% yield with steglich method. The chemical structure of the all the new synthesized valproic acid derivatives were characterized by FT-IR, NMR (HNMR, CNMR) and Mass Spectrometry. The synthesized amide derivatives of valproic acid evaluated for biological potential by subjecting to antibacterial activities for calculating the antibacterial potential of these compounds against bacteria. Bacterial strains used study against *Bacillis subtilis (+)* and *Escherichia coli (-)*. Ampicillin and ibuprofen were used standard drugs while ciprofloxacin used as positive control. The compound 3b exhibited the best antibacterial activity and showed the values MIC 2.0 mg/mL and 0.9mg/mL against *Bacillis subtilis (+)* and *Escherichia coli (-)* respectively. The compounds 3b and 3c maximum zone of inhabitation by depicting values 43mm against *Bacillis subtilis (+)* and 40mm against *Escherichia coli (-)* respectively. The results also showed that valproic acid derivatives not only anticonvulsant drug but also a good antibacterial agent.

Keywords: Valproic acid derivatives, VPA antibacterial activty, Valproic amide derivatives, Valproic acid

Valproic acid has significantly influenced drug development strategies within the pharmaceutical industry. Due to this reason, many biological activities have already been examined of VPA and its derivatives, like anti-bacterial, antiviral, and anti-cancers (Michaelis, Doerr, & Cinatl Jr, 2007; Zutz et al., 2016). The success has spurred further research into related compounds that can target similar pathways with potentially fewer side effects or improved efficacy. Moreover, the broad therapeutic potential of valproic acid has encouraged investigations into its use for diseases beyond its original applications, showcasing a model for repurposing existing drugs to expand their utility. Valproic acid's role in the pharmaceutical industry underscores the critical impact that specific chemical compounds can have on treatment paradigms and patient care (Mattson et al., 1985). It exemplifies how a single molecule can address complex, multifactorial conditions such as epilepsy and bipolar disorder, offering relief to millions of patients worldwide. As research continues, the potential for new applications of valproic acid remains a promising frontier in medicine, reflecting the dynamic and evolving nature of pharmaceutical science (Rogawski & Löscher, 2004).

Aniline derivatives occupy a significant role in the landscape of biological applications, where their chemical versatility is harnessed to address complex biological challenges. These compounds, through their variable structures, offer unique interactions within biological systems, facilitating advancements in both diagnostics and therapeutics. For example, some derivatives are crucial in the development of novel anti-malarial drugs, targeting parasite pathways that are critical for their survival and proliferation. Almost seventy five percent of nitrogen containing drugs, which are FDA approved available in the world markets (Kerru, Gummidi, Maddila, Gangu, & Jonnalagadda, 2020). The exploration of aniline derivatives represents a confluence of human curiosity and scientific rigor, aiming to extend the boundaries of our understanding and capability in improving human and environmental health (Anjalin, Kanagathara, & Suganthi, 2020).

Therefore, the aim of this research work to prepared different derivatives of VPA with anilines and then evaluates the biological activities of these synthesized derivatives.



Scheme 1: *Outline the synthesis of series of VPA derivatives with anilines 3a-f*

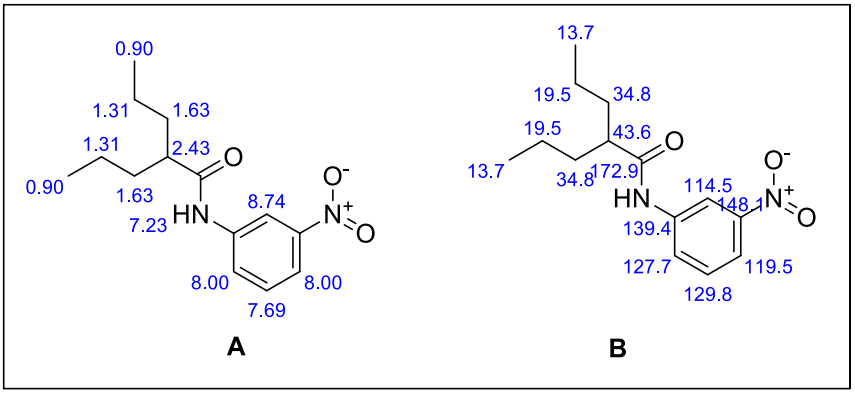


Fig. 1: *NMR Interpretation of 112b*

MATERIALS AND METHODS

General information

Each solvent and chemicals used of analytical grade were obtained from E. Merck Germany and underwent distillation at the minimum one time prior to use. Gallenkamp (melting apparatus) was used to know the MP (Melting point) of newly prepared VPA derivatives. The nuclear magnetic resonance spectrometer of model number AV-400 operated at frequency 500 megahertz to record the ^{1}H-NMR spectra. Similarly, the nuclear magnetic resonance spectrometer of model number AV-500 operated at frequency 100 megahertz to record the ^{13}C-NMR spectra. Chemical shifts were expressed in ppm (parts per million), while coupling constant J values were given in Hertz (Hz). FT-IR (Fourier transform-infra red) spectra were obtained using Bruker Fourier Transform IR spectrometer. Pre-coatedpolygram® SIL-G/UV 254 plates that were used in thin layer chromatography. The *fluka* company pre-coated polygram® SIL-G/UV 254 plates were available in commercial market Faisalabad, were used to detect reactants and products spots in the layer chromatography. A UV light was employed to identify the positions of spots during the experiments (Azizi, Aryanasab, & Saidi, 2006).

General Synthesis Procedure to Synthesis the amide derivative of VPA

The amide derivative of VPA aniline was prepared by dissolving the VPA (0.05g, 0.0003 moles) in DCM (10 ml) at 25^{o}C temperature at ice bath with constant magnetic stirring for 30 to 60 minutes. Then pyridine (0.127 ml, 0.00045 moles) was poured into the solution. Let the chemical dissolving for 25 to 30 minutes on cooling till completion. After on complete mixing with aniline was added. Again let the

reaction happened for 24 to 48 hours till completion. The condition of reaction was monitored and finalized through TLC. Upon completion of the synthesis reaction, the final product was obtained through filtration. The impure product was then purified by recrystallization using cool H_2O and n-hexane. After completing the crystallization, the product was dried and added in a glass vial and weighted to calculate the quantity of the aniline derivative of VPA (Ghosh & Shahabi, 2021).

Biological Evaluation

Antibacterial activity

To assess the antibacterial activity of these compounds, the disc diffusion method was employed. A 100 µL suspension of the microorganisms was spread onto nutrient agar using a sterilized loop. A specified concentration of the compound solution (0.05 mg per 100 µL) in chloroform was applied to filter paper discs, which were then placed on the agar plates pre-inoculated with bacterial cells. As a control, ciprofloxacin was applied to separate plates. After storing the plates at 4°C for 60 minutes, they were incubated at thirty seven degree centigrade for twenty four hours. The effectiveness of the treatments was measured by the diameter of the inhibition zones around the discs, as detailed by Aydinli et al. (2022).

Minimum Inhibitory Concentration (MIC)

To calculate the MIC values for various compounds, the resazurin microtiter-plate assay was employed. This assay involved preparing solutions of standard antibiotics and test compounds at concentrations of 10 mg/mL and 100 mg/mL in 10% DMSO. Approximately 100 µL of these solutions were added to the first row of a 96-well plate, with each subsequent row receiving 50 µL of a water bath. Then, 10 µL of both indicator resazurin and microbial suspensions were put to each well. The plates, which also included wells with solutions of new derivatives, 10% DMSO (negative control), ciprofloxacin (positive control), and microbial broth, were incubated at 37°C for 24 hours. Afterwards, the temperature was reduced to 25°C for an additional 48 hours. The change in color in the well plates was used to assess the MIC values, as documented by Khan, Ameen, Khan, Al-Arfaj, and Ahmed (2020).

Statistical analysis

Each experiment was conducted in triplicate, and the statistical analysis was performed in MS Excel 2013. The experiment data were presented as the mean ± standard deviation (SD) (Mcgrath et al., 2020).

Results and Discussion

Synthesis of aryl amide derivatives of VPA 3a-f

Coupling of valproic acid 2 with various substituted anilines (1a-f) using DCM in solvent at 25^0C conditions with pyridine, was carried out to afford aryl amide derivatives of VPA (3a-f) in excellent yields (85-91%) (Scheme 1) (Xie et al., 2019).

Antibacterial activity

Antibacterial activity of all the target molecules (3a-f) was carried out using disc diffusion method. Ampicillin and buprofen were used as standard drugs while ciprofloxacin used as positive control (Akhtar et al., 2023).

A compound Synthesis of 3-nitrophenyl-2-propylpentanamide (3b) have exhibited maximum zone of inhibition value (43mm) as compared to the standard drugs and positive control (29.2mm). However, compounds 3d, 3e and 3f depicted ZI values (34mm, 33mm and 25mm respectively) closure to ciprofloxacin. While no zone area was observed in case of 3a and 3c (0.4mm and 0.5mm respectively) showed poor anti-bacterial activity. In case of Escherichia coli, only compound 3c showed maximum zone of inhibition value (40mm) as

compared to standard drugs. 3a, 3b and 3f displayed antibacterial activity almost similar to ciprofloxacin by depicting Z1 value 13mm, 32mm and 12mm. While 3e (8.6mm) showed poor activity as compared to positive control and no zone was observed for compounds 3d (0.4mm) (Chai et al., 2011)

Minimum Inhibitory Concentration (MIC)

The compound 3b exhibited the best the values of MIC 2.0 mg/mL and 0.9 mg/mL against *Bacillis subtilis (+)* and *Escherichia coli (-)* respectively. The compounds 3a, 3c and 3e displayed almost same MIC values 2.4 mg/mL, 2.3mg/mL and 2.5mg/mL against *Bacillis subtilis (+)*, while the compounds 3d exhibited the least MIC 3.9mg/mL against *Bacillis subtilis(+)*. Against *Escherichia coli (-)* 3e dispalyed value (1.1mg/mL) almost near to compound 3b value (0.9mg/mL). The compounds 3c and 3f displayed almost same MIC values 1.5mg/mL, and 1.4mg/mL against *Escherichia coli (-)*, while the compounds 3a exhibited the least MIC 3.0 mg/mL against *Escherichia coli (-)* (Abdulah et al., 2017).

Spectral characterization of derivative 3b was conducted via FT-IR, ^{1}H-NMR and ^{13}C-NMR spectroscopy. In the FT-IR spectrum, characteristic absorption band of (nitrogen singal bond hydrogen) appears at 3422.03 cm^{-1}, while C=O exhibited peak at 1739.85 cm^{-1}, N=O appeared at 1494.9 and N-C 1270.94. Proton NMR spectrum of 3b gave triplet at 0.9 ppm for 6 hydrogens of VPA, however 4 protons of VPA appeared as broad Multiplets at 1.31 ppm and next 4 protons displayed the signal at 1.52 ppm of VPA because these protons found near to carbonyl group of VPA and a single proton of linker (methylene) appeared at 2.38 ppm. Singlet showed of the proton attached to the nitrogen of amine group. Doublet of doublet, doublet and singlet appeared at 7.6, 8.00 ppm and 8.74 ppm for four single hydrogens of phenyl ring respectively. Carbon NMR spectrum of 3b gave significant C=O peak at 172.9 ppm of carbonyl carbon, while VPA carbons (C-1, C-1'), (C-2,C-2'), (C-3,C-3'), (C-4), showed the peaks at 13.7, 19.5, 34.8 and 43.6 respectively. The C-5 to C-10 peaks, which appeared at from 119.5, 127.7, 129.6, 139.4 and 148.1, ppm respectively represents carbons associated with phenyl. All the other VPA aniline prepared derivatives molecules were elucidated in the same protocol.

Table 1: Spectral studies of newly prepared VPA derivatives (3a-f)

Compound	MP (°C)	Yield (%)	FT-IR (cm^{-1}) ν_{max} / ^{1}HNMR (500 MHz, CDCl3)/ ^{13}C NMR (100 MHz, $CDCl_3$)/ MS (EI) (m/z)
3a	195	90	(N-H); 3371.2, (C-H); 3188.8, (C=O); 1728.2, (C=C); 1637.4, (C-C); 1621.8, (N=O); 1448.1, (N-C); 1219.1, ^{1}H NMR (δ) Hz: 0.9 (t, 6H, J = 6.5, VPA), 1.31 (m, 4H, J = 6.5, VPA), 1.63 (q, 4H, J = 6.5, VPA), 2.43 (m, 1H, J = 7.6, VPA), 7.23 (s, 1H, NH) , 7.78 (t, 1H, J = 8.4, Ph-H), 7.82 (t, 1H, J = 8.4, Ph-H), 8.08 (d, 1H, Ph-H), 8.24 (d, 1H, Ph-H). 13C NMR(δ): 13.7 ($2CH_3$, VPA), 19.5 ($2CH_2$, VPA), 34.8 ($2CH_2$, VPA), 43.6 (CH, VPA), 119.5, 127.7, 129.6, 139.4, 148.1, (Ph-C), 172.9 (C=O).
3b	198	85	(N-H); 3422.03, (C-H); 3320.01, (C=O); 1739.85, (C=C); 1649.7, (C-C); 1625.66, (N=O); 1494.9, (N-C); 1270.94. ^{1}H NMR (δ) Hz: 0.9 (t, 6H, J = 6.5, VPA), 1.31 (m, 4H, J = 6.5, VPA), 1.63 (q, 4H, J = 6.5, VPA), 2.43 (m, 1H, J = 7.6, VPA), 7.23 (s, 1H, NH) , 7.69 (t, 1H, J = 8.4, Ph-H), 8.00 (d, 2H, Ph-H), 8.74 (s, 1H, Ph-H). ^{13}C NMR(δ): 13.7 ($2CH_3$, VPA), 19.5 ($2CH_2$, VPA), 34.8 ($2CH_2$, VPA), 43.6 (CH, VPA), 114.5, 119.5, 127.7, 129.8, 139.4, 148.1, (Ph-C), 172.9 (C=O).
3c	150	87	(N-H); 3320.87, (C-H); 3065.08, (C=O); 1739.85, (C=C); 1608.6,(C-C); 1621.0, (N-C); 1270.89, (C-Br); 1116.13, ^{1}H NMR (δ) Hz: 0.9 (t, 6H, J = 6.5, VPA), 1.31 (m, 4H, J = 6.5, VPA), 1.63 (q, 4H, J = 6.5, VPA), 2.43 (m, 1H, J = 7.6, VPA), 7.23 (s, 1H, NH) , 7.92 (s, 2H, J = 8.4, Ph-H). ^{13}C NMR(δ): 13.7 ($2CH_3$, VPA), 19.5 ($2CH_2$, VPA), 34.8 ($2CH_2$, VPA), 43.6 (CH, VPA), 115.0, 127.0, 127.6, 146.4, (Ph-C), 172.9 (C=O).
3d	209	91	(N-H); 3325.7, (C-H); 3197.9, (C=O); 1735.3, (C=C); 1660.8, (C-C); 1659.0, (N=O); 1300.2, (N-C); 1284.7, ^{1}H NMR (δ) Hz: 0.9 (t, 6H, J = 6.5, VPA), 1.31 (m, 4H, J = 6.5, VPA), 1.63 (q, 4H, J = 6.5, VPA), 2.43 (m, 1H, J = 7.6, VPA), 7.23 (s, 1H, NH) , 8.07 (d, 1H, J = 8.4, Ph-H), 8.63 (d, 1H, Ph-H), 8.77 (s, 1H, Ph-H). ^{13}C NMR(δ): 13.7 ($2CH_3$, VPA), 19.5 ($2CH_2$, VPA), 34.8 ($2CH_2$, VPA), 43.6 (CH, VPA), 120.2, 123.4, 130.2, 137.4, 143.3, 144.4 (Ph-C), 172.9 (C=O)
3e	202	89	H); 3389.8, (C-H); 3197.5, (C=O); 1719.7, (C=C); 1629.9,(C-C); 1629.8, (N-C); 1344.4, (C-Cl); 1076.1, ^{1}H NMR (δ) Hz: 0.9 (t, 6H, J = 6.5, VPA), 1.31 (m, 4H, J = 6.5, VPA), 1.63 (q, 4H, J = 6.5, VPA), 2.43 (m, 1H, J = 7.6, VPA), 7.23 (s, 1H, NH) , 7.87 (s, 1H, J = 8.0, Ph-H), 7.97 (s, 1H, Ph-H). ^{13}C NMR(δ): 13.7 ($2CH_3$, VPA), 19.5 ($2CH_2$, VPA), 34.8 ($2CH_2$, VPA), 43.6 (CH, VPA), 124.8, 127.0, 128.8, 129.3, 130.4, 136.8 (Ph-C), 172.9 (C=O).
3f	135	85	(N-H); 3389.8, (C-H); 3197.5, (C=O); 1719.7, (C=C); 1629.9, (C-C); 1629.8, (N-C); 1344.4, (C-Cl); 1076.1, ^{1}H NMR (δ) Hz: 0.9 (t, 6H, J = 6.5, VPA), 1.31 (m, 4H, J = 6.5, VPA), 1.63 (q, 4H, J = 6.5, VPA), 2.43 (m, 1H, J = 7.6, VPA), 7.23 (s, 1H, NH) , 7.47 (d, 2H, J = 8.0, Ph-H), 7.75 (d, 2H, Ph-H). ^{13}C NMR(δ): 13.7 ($2CH_3$, VPA), 19.5 ($2CH_2$, VPA), 34.8 ($2CH_2$, VPA), 43.6 (CH, VPA), 120.4, 129.0, 133.3, 136.6 (Ph-C), 172.9 (C=O).

Table 2: *Antibacterial activity results of compounds 3a-f*

Compound	*Bacillus subtilis*		*Escherichia coli*	
	ZI [a] (mm)	MIC [a] (mg/ml)	ZI [a] (mm)	MIC [a] (mg/ml)
3a	0.4 ± 0.44	2.4 ± 0.44	13 ± 0.44	3.0 ± 0.44
3b	43 ± 0.45	2.0 ± 0.45	32 ± 0.45	0.9 ± 0.45
3c	0.5 ± 0.68	2.5 ± 10.68	40 ± 0.68	1.5 ± 0.68
3d	34 ± 0.88	3.9 ± 0.88	0.4 ± 0.88	1.9 ± 0.88
3e	33 ± 0.78	2.3 ± 0.78	8.6 ± 0.78	1.1 ± 0.78
3f	25 ± 0.99	3.5 ± 0.99	12 ± 0.99	1.4 ± 0.99
Ampicillin	18 ± 1.02	10 ± 1.02	13 ± 0.08	16 ± 1.11
Iburofen	15 ± 1.00	14 ± 1.00	13 ± 1.00	21 ± 1.49
Ciprofloxacin	29.2 ± 1.9	1.0 ± 0.90	31.2 ± 1.2	2.4 ± 0.09

[a] Experiments were performed in triplicates and expressed as mean ± SD
* $p<0.05$, was considered significant

Conculsion

The activity of synthesized compounds **3a-f**, tested and these valproic acid derivatives showed high therapeutical potential against bacterial infections. While when the anilines acyl or aryl groups combine with the valproic acid, synthesized compounds showed higher and even better bacteriostatic activity as compared to the other synthesized valproic acid. As antibacterial activity, valproic derivatives found to be exceedingly potent. In the previous literature, the valproic based derivatives synthesized through multiple steps and time taking. As comparison to the reaction conditions of already synthesized derivatives, the new aniline-VPA derivatives **3a-f**, synthesize using single step with simple and easily available solvents. The synthesize VPA-aniline derivatives afford in better yield and in lesser time. The compound 3b exhibited the best antibacterial activity and showed the values MIC 2.0 mg/mL and 0.9mg/mL against *Bacillis subtilis (+)* and *Escherichia coli (-)* respectively. The compounds 3b and 3c maximum zone of inhabitation by depicting values 43mm against *Bacillis subtilis (+)* and 40mm against *Escherichia coli (-)* respectively. The results also showed that valproic acid derivatives not only anticonvulsant drug but also a good antibacterial agent.

References

Abdulah, R., Milanda, T., Sugijanto, M., Barliana, M. I., Diantini, A., Supratman, U., & Subarnas, A. (2017). Antibacterial properties of selected plants consumed by primates against Escherichia coli and Bacillus subtilis. *Southeast Asian J. Trop. Med. Public Health*, *48*(1), 109-116.

Akhtar, R., Zahoor, A. F., Mansha, A., Kamal, S., Khan, S. G., Raza, Z., & Ali, K. G. (2023). Protease inhibition, in vitro antibacterial and IFD/MM-GBSA studies of ciprofloxacin-based acetanilides. *PLoS One*, *18*(3), e0281044. doi:10.1371/journal.pone.0281044

Anjalin, M., Kanagathara, N., & Suganthi, A. B. (2020). A brief review on aniline and its derivatives. *Materials Today: Proceedings*, *33*, 4751-4755.

Aydinli, S. G., Bulut, E., Deniz, N. G., Sayil, C., Komarovska-Porokhnyavets, O., Lubenets, V., . . . Kravchenko, I. (2022). New Ketene Dithioacetals Generated from 2-Nitroperchlorobutadiene and Investigation of Their Antibacterial, Antifungal, Anticonvulsant and Antidepressant Activities. *Chemistry & Biodiversity*, *19*(7), e202100931.

Azizi, N., Aryanasab, F., & Saidi, M. R. (2006). Straightforward and highly efficient catalyst-free one-pot synthesis of dithiocarbamates under solvent-free conditions. *Org Lett*, *8*(23), 5275-5277. doi:10.1021/ol0620141

Chai, Y., Liu, M. L., Lv, K., Feng, L. S., Li, S. J., Sun, L. Y., . . . Guo, H. Y. (2011). Synthesis and in vitro antibacterial activity of a series of novel gatifloxacin derivatives. *Eur J Med Chem*, *46*(9), 4267-4273. doi:10.1016/j.ejmech.2011.06.032

Ghosh, A. K., & Shahabi, D. (2021). Synthesis of amide derivatives for electron deficient amines and functionalized carboxylic acids using EDC and DMAP and a catalytic amount of HOBt as the coupling reagents. *Tetrahedron letters*, *63*, 152719.

Kerru, N., Gummidi, L., Maddila, S., Gangu, K. K., & Jonnalagadda, S. B. (2020). A Review on Recent Advances in Nitrogen-Containing Molecules and Their Biological Applications. *Molecules*, *25*(8). doi:10.3390/molecules25081909

Khan, A., Ameen, F., Khan, F., Al-Arfaj, A., & Ahmed, B. (2020). Fabrication and antibacterial activity of nanoenhanced conjugate of silver (I) oxide with graphene oxide. *Materials Today Communications*, *25*, 101667.

Mattson, R. H., Cramer, J. A., Collins, J. F., Smith, D. B., Delgado-Escueta, A. V., Browne, T. R., . . . et al. (1985). Comparison of carbamazepine, phenobarbital, phenytoin, and primidone in partial and secondarily generalized tonic-clonic seizures. *N Engl J Med*, *313*(3), 145-151. doi:10.1056/nejm198507183130303

Mcgrath, S., Zhao, X., Steele, R., Thombs, B. D., Benedetti, A., & Collaboration, D. S. D. (2020). Estimating the sample mean and standard deviation from commonly reported quantiles in meta-analysis. *Statistical methods in medical research*, *29*(9), 2520-2537.

Michaelis, M., Doerr, H. W., & Cinatl Jr, J. (2007). Valproic acid as anti-cancer drug. *Current pharmaceutical design*, *13*(33), 3378-3393.

Rogawski, M. A., & Löscher, W. (2004). The neurobiology of antiepileptic drugs. *Nature reviews neuroscience*, *5*(7), 553-564.

Xie, M. S., Zhang, Y. F., Shan, M., Wu, X. X., Qu, G. R., & Guo, H. M. (2019). Chiral DMAP-N-oxides as acyl transfer catalysts: Design, synthesis, and application in asymmetric steglich rearrangement. *Angewandte Chemie*, *131*(9), 2865-2869.

Zutz, C., Bacher, M., Parich, A., Kluger, B., Gacek-Matthews, A., Schuhmacher, R., . . . Strauss, J.

(2016). Valproic acid induces antimicrobial compound production in Doratomyces microspores. *Frontiers in microbiology*, *7*, 510.