Synthesis of a new drug candidate trough molecular hybridization and application as a learning strategy

Síntese de um novo candidato à fármaco através de hibridação molecular e aplicação como estratégia de aprendizagem

DOI:10.34117/bjdv7n3-096

Recebimento dos originais: 08/02/2021
Aceitação para publicação: 01/03/2021

Leyliane Teixeira Dias dos Santos
Bacharel em Farmácia
Centro Universitário Autônomo do Brasil, UNIBRASIL
Endereço: R. Konrad Adenauer, 442 - Tarumã, Curitiba - PR, 82820-540, Brazil
Email: leylianepma@hotmail.com

Mariana Lopes Teixeira
Mestra em Química
Centro Universitário Autônomo do Brasil, UNIBRASIL
Endereço: R. Konrad Adenauer, 442 - Tarumã, Curitiba - PR, 82820-540, Brazil
Email: maryanalt@gmail.com

Jaqueline Carneiro
Doutora em Ciências Farmacêuticas
Centro Universitário Autônomo do Brasil, UNIBRASIL
Endereço: R. Konrad Adenauer, 442 - Tarumã, Curitiba - PR, 82820-540, Brazil
E-mail: profjaquelinec@gmail.com

ABSTRACT

Introduction: Molecular hybridization is a synthesis strategy in which new molecules are obtained by linking two drugs or pharmacophoric groups. Skin conditions treatment, such as acne, generally requires the association of drugs, for instance, sulfacetamide and salicylic acid. The present study aimed to synthesize and characterize a new drug candidate by molecular hybridization using sulfacetamide and salicylic acid. We also intended to further explore and evaluate this synthesis as a practical educational strategy for undergraduate students. Methods: The synthesis was carried out through an azo coupling reaction between the aromatic amine from sulfacetamide and the ring from salicylic acid. The compound obtained was characterized by melting point and infrared spectroscopy (IR). A short course was developed and applied to evaluate the impact of this synthesis and its discussion in class. The evaluation was performed using an initial and a final questionnaire. Results: The compound was obtained as an orange solid (41%), with a melting point of 108 ºC, and showing as main IR bands: 3247-3041 cm^{-1}, 1662 cm^{-1}, 1608 cm^{-1}, 1439 cm^{-1}. The data from the questionnaires showed that the students improve their knowledge of drug development strategies (73%). Discussion: The experimental evidences suggest the formation of a new chemical entity, possibly the proposed hybrid. Besides, the questionnaire results suggest that new teaching methodologies mixing theoretical-practical moments, allowed students to better understand a specific issue. Conclusion: The proposal synthesis showed to be viable and

an interesting possibility to approach several issues with pharmacy undergraduate students in a multidisciplinary way.

Keywords: organic synthesis, diazotization, short course, pharmacy education.

RESUMO

Introdução: A hibridação molecular é uma estratégia de síntese em que novas moléculas são obtidas ligando dois medicamentos ou grupos farmacópicos. O tratamento de condições de pele, como a acne, requer geralmente a associação de drogas, por exemplo, sulfacetamida e ácido salicílico. O presente estudo visava sintetizar e caracterizar um novo candidato a fármaco através da hibridação molecular utilizando sulfacetamida e ácido salicílico. Pretendemos também explorar e avaliar melhor esta síntese como uma estratégia educacional prática para estudantes de graduação. Métodos: A síntese foi realizada através de uma reacção de acoplamento azo entre a amina aromática da sulfacetamida e o anel do ácido salicílico. O composto obtido foi caracterizado por espectroscopia de ponto de fusão e infravermelho (IR). Foi desenvolvido e aplicado um pequeno curso para avaliar o impacto desta síntese e a sua discussão na aula. A avaliação foi realizada utilizando um questionário inicial e um questionário final. Resultados: O composto foi obtido como um sólido laranja (41%), com um ponto de fusão de 108 ºC, e mostrando como principais bandas IR: 3247-3041 cm-1, 1662 cm-1, 1608 cm-1, 1439 cm-1. Os dados dos questionários mostraram que os estudantes melhoram os seus conhecimentos sobre estratégias de desenvolvimento de fármacos (73%). Discussão: As evidências experimentais sugerem a formação de uma nova entidade química, possivelmente o híbrido proposto. Além disso, os resultados do questionário sugerem que novas metodologias de ensino que misturam momentos teórico-práticos, permitiram aos estudantes compreender melhor uma questão específica. Conclusão: A síntese da proposta mostrou ser viável e uma possibilidade interessante de abordar várias questões com estudantes universitários de farmácia de uma forma multidisciplinar.

Palavras-chave: síntese orgânica, diazotização, curso curto, educação em farmácia.

1 INTRODUCTION

The emergence of new synthetic methodologies and new drugs after the industrial and technological revolution of the 19th century contributed significantly to the increase in human survival.[1] Since then, the pharmaceutical industry has made available several therapeutic options to expand the population's access to the most varied health treatments. Still, the medications made available are progressively more effective and safer.[2]

Among the various methodologies for obtaining new drug candidates, the synthesis by molecular hybridization (HM) is a classic strategy and of great interest for the pharmaceutical industry. The new molecules obtained, called hybrids, are the result of the synthesis process in which there is a connection between drugs or pharmacophoric groups, which can generate molecules with synergistic actions when compared with the starting drugs.[3] Also, HM starts from molecules with known physical-chemical,

pharmacological, and toxicity characteristics, making it possible to develop chemical libraries with numerous distinct hybrids with high potential as new candidates for new drugs.[4,5]

Previous studies have reported several promising chemical compounds with anticancer activity[3] and also cardioactive, anti-inflammatory, antithrombotic, antibacterial and analgesic agents, obtained through the technique of HM.[6] Araújo et al., 2015, portrayed the hybridization of paracetamol molecules (drug of analgesic and antipyretic activity) and sulfadiazine (antimicrobial drug) to obtain hybrids with potential analgesic, antipyretic and antimicrobial synergistic effect.[7]

The treatment of skin conditions, such as acne, can be quite complex and require a combination of different pharmaceutical ingredients. HM strategy is interesting in this case since two drugs are widely used in this regard: sulfacetamide and salicylic acid. Sulfacetamide is a sulfonamide antimicrobial with antiseborrheic action.[8,9], while salicylic acid is a comedolytic and keratolytic agent.[10]

Since this proposed synthesis is of easy execution, unprecedented as far as we know, and has a high potential to attract students' attention, this study aimed to synthesize and characterize a new drug candidate using molecular hybridization of sulfacetamide and salicylic acid. Moreover, an experimental course related to the theme can explore synthetic strategies in drug development, molecular hybridization, diazotization reactions, reaction mechanisms, and characterization of organic compounds. Therefore, this work has also explored and evaluated the application of the studied synthetic methodology as a practical educational strategy.

2 MATERIAL AND METHODS

Sulfacetamide sodium and salicylic acid were obtained commercially. The solventes used, hydrochloric acid (6 $mol.L^{-1}$), sodium nitrite, and sodium hydroxide were all previously treated and distilled.

*Synthesis of 5-[4- (*N*-acetilsulfamoyl)phenyl]diazenyl]-2-hidroxybenzoic acid*

In a conical flask, under magnetic stirring, sulfacetamide (5 mmol, 1.27 g) and 7.5 ml of concentrated hydrochloric acid (6 $mol.L^{-1}$) were added. The mixture was cooled to 0 ºC and then an aqueous solution of $NaNO_2$ (2 mL; 1 $mol.mL^{-1}$) was added. Simultaneously, the diazonium salt previously formed was added drop by drop to a round bottom flask containing salicylic acid (5 mmol, 0.69 g) and 5 mL of a 10% sodium hydroxide solution (w/v). The system was kept in an ice bath (0 ºC) and stirring for 40

minutes. The flask was kept at room temperature for another 60 minutes. The mixture was filtered under a vacuum and the resulting solid was washed with 100 mL of distilled water and dried at room temperature.

DETERMINATION OF MELTING POINT

Melting point determination was performed by placing the sample in a capillary with one of the ends previously closed, then inserting it into the equipment (QUIMIS Q-340D23) for a gradual increase in temperature. The melting temperature was observed and noted. The procedure was carried out in triplicate.

INFRARED SPECTROSCOPY

For the acquisition of the infrared spectra, the starting materials and the hybrid were incorporated separately into KBr pellets and subsequently analyzed in a BOMEM MB100 spectrometer with Fourier transform, at a spectral range of 4000-400 cm^{-1} with 64 $scans.min^{-1}$, and resolution of 4 cm_{-1}. The procedure was performed in duplicate.

SHORT COURSE APPLICATION

A short course with twenty pharmacy undergraduate students was developed and performed. The three-hour course was split into theoretical and practical steps. In the beginning, an initial questionnaire was applied. The students were supposed to answer questions that evaluated their perception of the relevance of the theme; their notions of stoichiometry and yield calculation; knowledge of drug development strategies; perception of multidisciplinarity applications; and perception of an association between theory and practice during undergraduate school.

The students were divided into six teams of three or four individuals, instructed, and lead to the laboratory. Then, they performed the hybrid synthesis previously described. During the reaction period, the responsible teachers introduced and deepened the themes: drug development strategies, molecular hybridization, stoichiometry, yield calculations, diazotization reactions, and mechanisms. Following, the students completed the reaction and the mixture was vacuum filtered. The resulting solid was washed with distilled water and dried in the oven at 50 ° C for 30 min. The infrared spectra and the melting point value were made available for interpretation and discussion. At the end of the course, the students answered the same questionnaire from the beginning.

3 RESULTS

The product was obtained as an orange solid (41% yield). The melting point found for the new compound was 108 ºC, and the main IV bands observed were 3247-3041 cm^{-1}, 1662 cm^{-1}, 1608 cm^{-1}, 1439 cm^{-1}, 1312 cm^{-1}.

In the evaluation of the short course applied questionnaires, it was possible to observe a positive impact on the development of the participants (Figure 1). There was a 10% increase in the perception of the relevance of organic synthesis in the scope of pharmaceutical sciences and the understanding of stoichiometry, as well as 45% for calculating reaction yield. Only 10% of students declared to know some drug development strategy at the beginning of the course, which went up to 83% at the end. 58% of the participants cited molecular hybridization as an example. Still, 100% of students responded assertively regarding the course's contribution to their training and highlighted the greater learning of theories when related to practices.

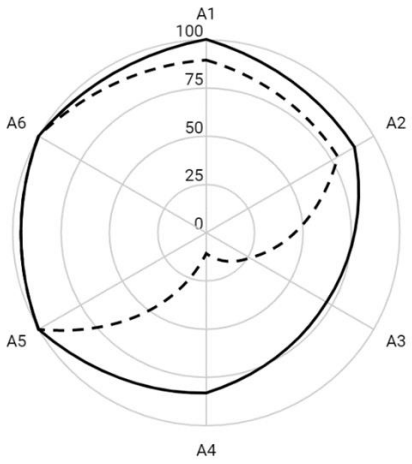

Figure 1. Results obtained from the questionnaires applied at the beginning of the course (---) and at the end (−). A1 = perception of the relevance of the theme; A2 = notions of stoichiometry; A3 = notions of yield calculation; A4 = knowledge of drug development strategies; A5 = perception of multidisciplinarity applications; A6 = perception of an association between theory and practice during undergraduate school.

4 DISCUSSION

The synthesis of hybrid compounds is one of the synthetic strategies more explored in the development of new drugs. The chemical structure of the hybrid is directly related to its biological activity and often reflects the properties of its original drugs.[11,12] In this study, an unprecedented hybrid compound obtained from sulfacetamide and salicylic acid, with high potential for application in dermatological conditions, was selected for the study of its synthetic route, characterization, and subsequent

incorporation. Also, given the possibility of synthesizing a new drug through a synthetic route accessible to higher education chemistry laboratories, the possibility and the impact of using the hybrid's synthetic methodology as an educational strategy in the pharmacy school was investigated.

The synthesis of the compound 5-[4-(*N*-acetilsulfamoyl)phenyl]diazenyl]-2-hidroxybenzoic acid was carried out in two steps, the first being the generation of the arenodiazonium salt (step 1) and the second to the formation of the corresponding hybrid between sulfacetamide and salicylic acid (step 2) (Figure 2).

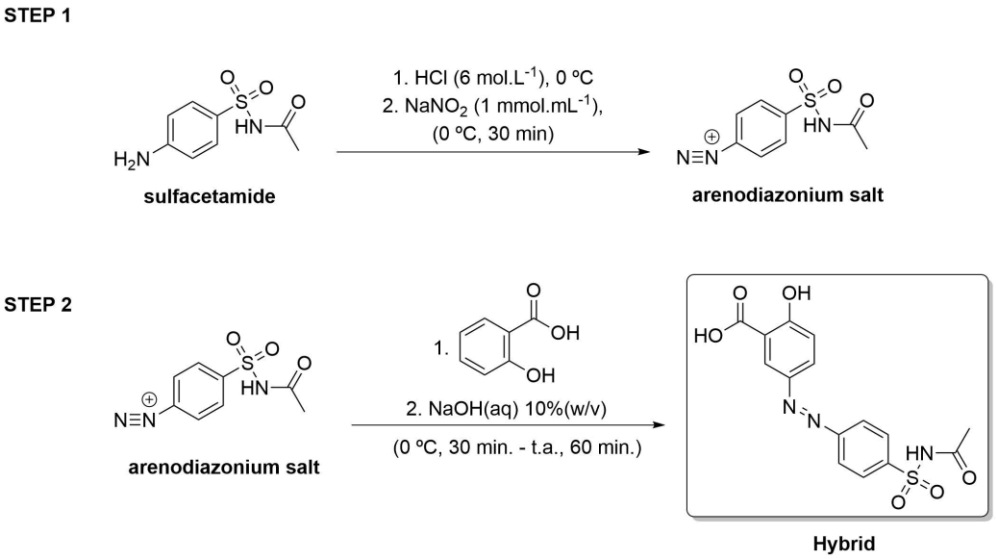

Figure 2. Synthetic route to obtain the hybrid between sulfacetamide and salicylic acid.

To prepare the arenodiazonium salt, sulfacetamide reacted with nitrous acid, from the reaction between hydrochloric acid and sodium nitrite. Then, salicylic acid, in a basic medium, was added to the intermediate through a substitution reaction. Hybrid purification was carried out using vacuum filtration and subsequent washing with distilled water.

Diazotization, also known as a coupling reaction, is the name given to the chemical reaction between amine groups and acid nitrous for the formation of a diazonium salt. According to the literature, nitrous acid can react with all classes of amines. In this sense, consequently, the product of a reaction of oxidation of amines with nitrous acid will depend on the choice of the class of amines used (primary, secondary, tertiary, aliphatic, or aromatic).[13,14] Sulfacetamide is a polyfunctionalized compound and, which contains in its structure a group primary amino attached to an aromatic ring. For

this reason, sulfacetamide, in the presence of nitrous, can generate a salt that acts as starting material for the synthesis of hybrid of interest, as seen in Figure 3A.

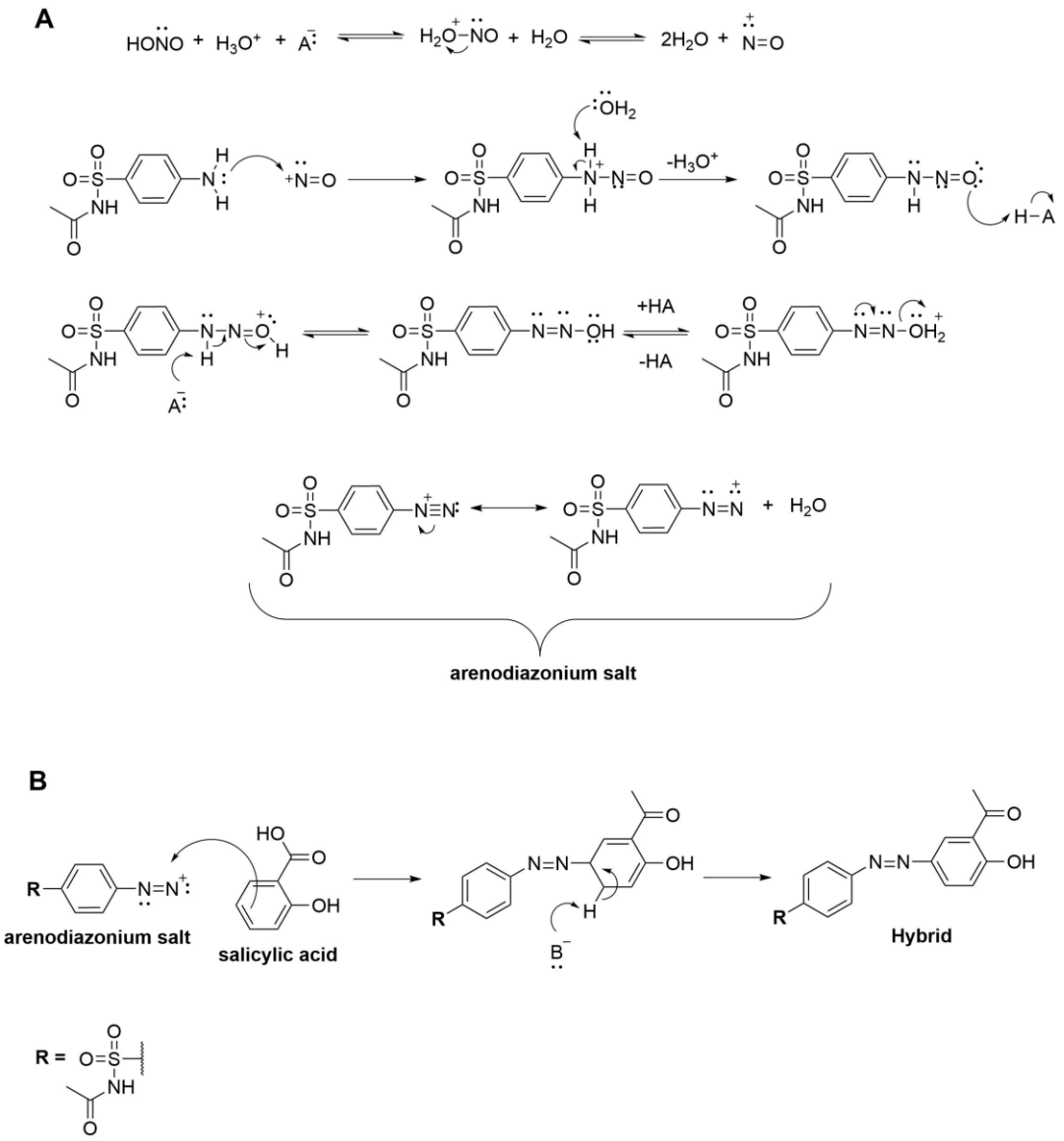

Figure 3. Diazotization mechanism (A) and molecular hybridization mechanism (B).

The importance of diazotization reactions in primary arylamines should be highlighted due to their considerable synthetic importance since the diazonium group can be replaced by a variety of other functional groups.[13,14] Therefore, the formation of arenodiazone salt in the sulfacetamide is essential to allow the hybridization with the

salicylic acid (Figure 3B).

The generation of a new N=N, characteristic of an azocomposite, facilitated the characterization of the product. The comparison of the bands of the starting materials with the product reveals new bands (Figure 4). It was possible to observe a new N=N band,

characteristic of azo compounds. The vibration suggested in the literature for azocomposites may vary according to the configuration shown (cis or trans). The cis and trans isomers generally have different absorption spectra that can vary from 1300 to 1600 cm^{-1}. The main bands observed were at 3300 - 3000 cm^{-1} (presence of hydroxyl), 1700-1600 cm^{-1} (presence of the carbonyl group), and 1608 cm^{-1} (N-H amide bond), 1492 cm^{-1} (N=N azo bond).[15]

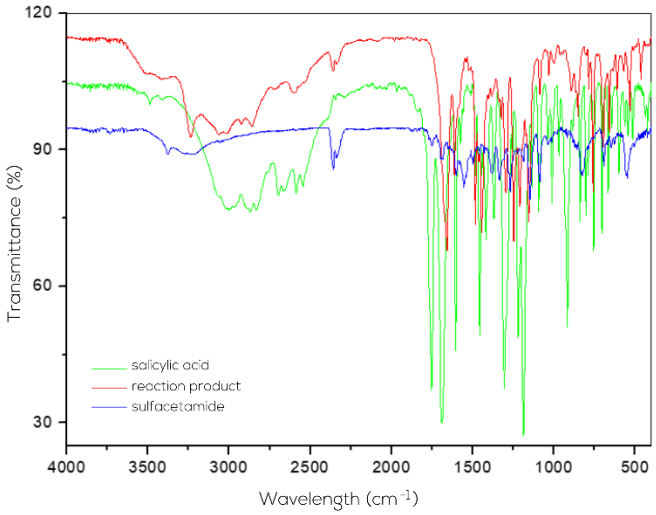

Figure 4. Comparison of the IR bands of the starting materials and the reaction product.

The melting point obtained for the product (108 °C) is different from the ones for the starting materials salicylic acid (156-160 °C) and sulfacetamide (180-184 °C).[16,17] This fact suggested the obtention of a new molecule.

The application of the previously mentioned syntheses as a short course showed to be doable and compelling. It was observed the engagement of the participants carrying the synthesis and all teams obtained the expected orange solid. By analyzing the applied questionnaires, it was possible to observe a positive impact on the participants' development. The application of the course showed to be of great potential as a learning strategy in the undergraduate course in Pharmacy, as well as a great opportunity to combine theory, practice, and multidisciplinarity approach.

5 CONCLUSION

The experimental product and the analysis of the compound suggest the formation of a new chemical entity, possibly the hybrid generated between sulfacetamide and salicylic acid. This result highlights the possibility of using a relatively simple synthesis,

with few steps, including purification, to obtain a new drug candidate. In this case, a new drug to treat skin conditions, such as acne. The experimental protocol applied as a learning tool showed to be promising. The questionnaire results suggest that new teaching methodologies mixing theoretical-practical moments, allows students to better understand a study topic. Therefore, the proposal synthesis showed to be viable and an interesting possibility to approach several issues with pharmacy undergraduate students in a multidisciplinary way.

DECLARATION OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

1 Nicolaou K, Rigol S. A brief history of antibiotics and select advances in their synthesis. J Antibiot. 2018;71:153–184.

2 Moore TJ, Psaty BM, Furberg CD. Time to act on drug safety. JAMA. 1998;279(19):1517-1573.

3. Viegas-Junior C, Danuello A, Bolzani VS, Barreiro EJ, Fraga CAM. Molecular hybridization: a useful tool in the design of new drug prototypes. Cur Med Chem. 2007;14:1829–1852.

4. Li H, Yu S, Fan T, Zhong Y, Gu T, Wu W. et al. Design, synthesis, and biological activity evaluation of BACE1 inhibitors with antioxidant activity. Drug Dev Res. 2020;81(2):206-214.

5. Rajesh AR, Vikas NT. Synthesis and evaluation of novel chloropyrrole molecules designed by molecular hybridization of common pharmacophores as potential antimicrobial agents. Bioorg Med Chem Lett. 2010;20(19):206-214.

6. Lazar C, Kluczyk A, Kiyota T, Konishi Y. Drug Evolution Concept in Drug Design: 1. Hybridization Method. J Med Chem. 2004;47(27):6973-6982.

7. Araujo CR, Filho CAL, Santos VLA, Maia GLA, Gonsalves AA. Desenvolvimento de fármacos por hibridação molecular: uma aula prática de química medicinal usando comprimidos de paracetamol e sulfadiazina e a ferramenta virtual SciFinder®. Quím Nova. 2015;38:868-873.

8. Nagaraja P, Sunitha KR, Vasantha RA, Yathirajan HS. Iminodibenzyl as a novel coupling agent for the spectrophotometric determination of sulfonamide derivatives Eur J *Pharm Biopharm.* ***2002;****53:*187–192.

9. Torres DR, Sosnik A, Chiappetta D, Vargas EF, Martínez F. Entalpía de disolución de sulfacetamida sódica en agua: comparación entre la calorimetría isoperibólica de solución y el método de Van't Hoff. *Quím Nova.* ***2008****;31:*1455-1459.

10. Lee H-S, Kim I-H. Salicylic acid peels for the treatment of acne vulgaris in Asian patients. Dermatol Surg. 2003;29(12):1196-9.

11. Ivasiv V, Albertini C, Gonçalves AE, Rossi M, Bolognesi ML. Molecular hybridization as a tool for designing multitarget drug candidates for complex diseases.

Curr Top Med Chem. 2019;19(19):1694-1711.

12. Bosquesi PL, Melo TRF, Vizioli EO, Santos JL, Chung MC. Anti-inflammatory drug design using a molecular hybridization approach. *Pharmaceuticals.* **2011;***4:*1450-1474.

13. Butler NR. Diazotization of heterocyclic primary amines. Chem Rev. 1975;75(2):241–257.

14. Liu Y, Zeng C, Wang C, Zhang L. Continuous diazotization of aromatic amines with high acid and sodium nitrite concentrations in microreactors. J Flow Chem. 2018;8:139-146.

15. Chen S, Chen X, Xia T, Ma Q. A novel electrochemiluminescence sensor for the detection of nitroaniline based on the nitrogen-doped graphene quantum dots. Biosens Bioelectron. 2016;85:. 10.1016/j.bios.2016.06.010.

16. Nagula RG, Khan RA, Nanjia A. Modulating the solubility of sulfacetamide by means of cocrystals. Cryst Eng Comm. 2014;16(26):5859.

17. Bica K, Rijksen C, Nieuwenhuyzen M, Rogers RD. In search of pure liquid salt forms of aspirin: ionic liquid approaches with acetylsalicylic acid and salicylic acid. Phys Chem Chem Phys. 2010;12:2011-2017.