N,N-dimethylacetamide. A versatile solvent for pharmacological applications: anti-thyroid activity?

Nancy Martini, Patricia A.M. Williams, Evelina G. Ferrer*
Centro de Química Inorgánica (CEQUINOR-CONICET-CICPBA-UNLP)-Departamento de Química- Facultad de Ciencias Exactas, Universidad Nacional de La Plata, Boulevard 120 entre 60 y 64, C.C.962- (B1900AVV) -1900 La Plata, Argentina.

N, N-dimetilacetamida. Un solvente versátil para aplicaciones farmacológicas: ¿actividad antitiroidea?

N, N-dimetilacetamida. Un solvent versàtil per a aplicacions farmacològiques: activitat antitiroidea?

RECEIVED: 2 DECEMBER 2020; ACCEPTED: 21 MAY 2021

ABSTRACT

N,N-dimethylacetamide is an industrialized solvent highly miscible with water, very useful in the pharmacological and chemical areas. This well-known solvent possesses great therapeutic potential. Its use has been proven in the treatment of inflammatory-infective diseases together with its antibacterial effect and bone regeneration. In this short communication, its potential anti-thyroid activity is presented. The experiment is based on the interference with iodine incorporation into the tyrosine residues of thyroglobulin and avoids the biosynthesis of thyroid hormones. The interaction with iodine was evaluated by the Benesi-Hildebrand method. It was found that *N,N*-dimethylacetamide was able to form a charge-transfer complex with iodine with a K_{CT} value higher than 100 M^{-1} which is a reference value for anti-thyroid activity. FTIR spectroscopy supports the formation of the charge-transfer complex which is assumed to occur via C=O--iodine interaction.

Keywords: Anti-thyroid activity; FTIR spectroscopy; *N,N*-dimethylacetamide; Pharmaceutical solvent.

RESUMEN

La N,N-dimetilacetamida es un solvente industrializado altamente miscible con agua, muy útil en el área farmacológica y química. Este conocido disolvente posee un gran potencial terapéutico. Su uso ha sido probado en el tratamiento de enfermedades inflamatorias-infecciosas junto con su efecto antibacteriano y como regenerador óseo. En esta breve comunicación se presenta su potencial actividad antitiroidea. El experimento se basa en interferir la incorporación de iodo en los residuos de tirosina de la tiroglobulina y evitar la biosíntesis de las hormonas tiroideas. La interacción con el iodo se evaluó mediante el método de Benesi-Hildebrand. Se encontró que la N,N-dimetilacetamida es capaz de formar un complejo de transferencia de carga con el iodo con un valor de K_{CT} mayor a 100 M^{-1}, el cual es un valor de referencia para la actividad antitiroidea. La espectroscopía FTIR confirma la formación del complejo de transferencia de carga que ocurre a través de la interacción C=O---iodo.

Palabras Claves: Actividad antitiroidea, Espectroscopía Infrarroja, N,N dimetilacetamida, solvente farmaceútico.

RESUM

La N,N-dimetilacetamida es un solvent industrialitzat altament miscible amb aigua, molt útil a l'àrea farmacològica i química. Aquest conegut dissolvent te un gran potencial terapèutic. El seu us ha sigut provat en el tractament d'infermetats inflamatòries- infeccioses junt amb el seu efecte antibacterià i com regenerador ossi. En aquest breu comunicat es presenta la seva potencial activitat antitiroidea. L'experiment es basa a interferir la incorporació de iode en els residus de tirosina de la tiroglobulina i evitar la biosíntesis de hormones tiroidals. La interacció amb el iode es va avaluar mitjançant el mètode de Benesi-Hildebrand. Es va trobar que la N,N-dimetilacetamida es capaç de formar un complex de transferència de carrega amb el

***Corresponding author: evelina@quimica.unlp.edu.ar**

iode amb un valor de KCT mes gran que 100 M-1, el qual es un valor de referencia per la activitat antitiroidea. L'espectroscòpia FTIR confirma la formació del complex de transferència de carrega que te lloc mitjançant la interacció C=O---iode.

Paraules clau: Activitat antitiroidea, Espectroscòpia Infraroja, N,N dimetilacetamida, solvent farmacèutic.

INTRODUCTION

N,N-dimethylacetamide (DMA) is a known industrial solvent very miscible with water, alcohol, and many organic solvents including mineral oil. This solvent, together with ethanol, n-propanol, isopropanol, N,N-dimethylformamide, polyethylene glycol, dimethyl sulfoxide is usually used for the solvency approach in the pharmaceutical field. Those solvents have all the required physicochemical properties (thermic stability, nontoxicity, noncorrosive and commercial availability) to be used as a vehicle in pharmaceutical formulations.[1] In particular, some examples of the rich chemistry of N,N-dimethylacetamide include: (i) organogels for cosmetic and dermo-cosmetic applications,[2] (ii) polymorphs crystallization and co-crystals,[3] (iii) optimization of poorly water-soluble substances,[1,4] (iv) antimicrobial sutures,[5] (v) tetracycline antibiotic compositions,[6] (vi) suitable penetration enhancing agent in topical pharmaceutical compositions,[7] and metal complexes.[8]

N,N-dimethylacetamide possess a background of therapeutic potential. Topical pharmaceutical formulations have been developed being the solvent the main active ingredient. In particular, a composition for anti-inflammatory treatment, especially those that come from inflammatory-infective illnesses wherein its antibacterial effect would be highly appreciated.9 This compound has been also proposed for the treatment of inflammatory disorders comprising: preterm birth and labor,10 cardiovascular, musculoskeletal, gastrointestinal, pulmonary, neurodegenerative, genitourinary autoimmune, oncologic, endocrine disorders and dermatologic diseases and infections.11 It has been also suggested that DMA enhances bone regeneration, binds bromodomains, and inhibits osteoclastogenesis. Therefore, it is proposed that it can behave as an anti-osteoporotic agent.[12,13] Finally, some findings showed that DMA has a strong potential to control adiposity and prevent the increment of fat-release being a potential drug candidate to prevent obesity.[14]

In the course of our investigations,[15,16] we have been working with substances with potential anti-thyroid activity looking for particular chemical characteristics such as the presence of NH or C=O chemical groups in the structure of the molecule. From the chemical point of view, DMA has two potential coordination points, namely through the oxygen or the nitrogen atoms, and also presents resonance structures. Previous research papers showed the ability of some drugs to act as anti-thyroid agents in special if they were able to interact with iodine *via* charge-transfer complex formation. In this sense, substances containing NH or C=O chemical groups, or both of them, have been successfully tested for their ability to interact with iodine such as antidepressants[15,17] and anti-thyroid drugs,[18,19] chloropurine,[20] and thiazolidine-2-thione,[21] among others. Those results motivated us to study DMA as a potential anti-thyroid agent through the analysis of its iodine interaction using UV-Vis and FTIR spectroscopies.

MATERIALS AND METHODS

All chemicals used were of analytical grade. Infrared solution spectra of DMA-I_2 (CCl_4) system were measured with a Bruker IFS 66 FTIR-spectrophotometer from 1700 to 1300 cm^{-1}. UV-Vis determinations were recorded with a Shimadzu 2600/2700 spectrophotometer.

In vitro anti-thyroid activity

Iodine complexation was assayed based on the Benesi-Hildebrand equation.[22] Iodine was bi-sublimated and kept in the dark in a desiccator containing P_2O_5. A spectroscopic grade solvent CCl_4 was used. Solutions of iodine and DMA were prepared just before the beginning of the experiments. Iodine concentration (acceptor) was kept at a constant concentration (4×10^{-4} M, CCl_4). For the spectrophotometric titration measurements a set of concentrations of DMA (donor) from 4×10^{-5} M to 1.5×10^{-3} M (CCl_4) were used and the stoichiometry of the CT-complex was calculated from the determination of the conventional spectrophotometric molar ratio using a plot of absorbance vs [donor/acceptor] of the CT-complex. The absorbance was measured at 515 nm. For the determination of the equilibrium constant of the donor:acceptor complex, a 0.01 M-0.05 M concentration range of DMA was used. The reaction was carried out directly in the spectrophotometer cell by mixing 1.5 mL solutions of the donor and the acceptor (iodine). Spectra were recorded immediately on a double beam UV-Vis spectrophotometer. The temperature of the solutions was kept during the measurements at 25 ± 1ºC. Three independent replicates of each solution were measured.

Besides, the free energy change ΔG^o was calculated from Gibbs free energy of formation according to the equation: $\Delta G^o = -RT \ln K_{CT}$, where ΔG^o is the free energy change of the CT-complex, R is the gas constant (8.31 J/Kmol), T is the temperature in Kelvin and K_{CT} is the formation constant of donor-acceptor complex. The energy of the charge-transfer complex was assumed as $E_{CT} = (h\nu_{CT}) = 1243.667/ \lambda_{CT}$ (eV), where λ_{CT} is the band wavelength of the complex in nm.[22]

RESULTS AND DISCUSSION

The formation of charge-transfer (CT) complexes has a great interest in pharmaceutical analysis because of their utility for the accessibility and rapid detection of many drugs and also for the study of drug-receptor mechanism.[23] The molecular interactions between

electron-donors and acceptors-molecules are usually related to the formation of an intense colored CT-complex which absorbs radiation in the visible. Particularly, the formation of charge-transfer complexes between anti-thyroid drugs and iodine is an important area of study for hyperthyroidism. The relevance is based on the chance to study one of the possible mechanisms of action of anti-thyroid drugs. It is proposed that in a first step, the thyroid peroxidase enzyme (TPO) is oxidized *via* the action of endogenous H_2O_2; in a second step, the oxidized TPO reacts with iodine to form MIT (monoiodotyrosine) and DIT (diiodotyrosine) (Figure 1) and then, in a successive intra-molecular rearrangement and coupling of the molecules the L-tri-iodothyronine (T_3) and L-tetra-iodothyronine or thyroxine (T_4) are formed. Hyperthyroidism condition is produced by the overproduction of thyroid hormones.

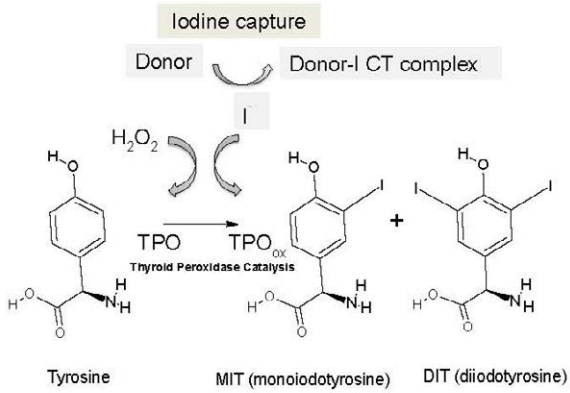


Figure 1. *Scheme of the plausible mechanism involved in the anti-thyroid activity.*

This overproduction can be avoided by (i) blocking the biosynthesis of thyroid hormone by the inhibition of the thyroid peroxidase (TPO) enzyme, (ii) acting on the H_2O_2 substrate, or (iii) blocking the iodination process by forming stable CT-complex with iodine. It is known that several substances having an electron lone-pair on the oxygen, sulfur, and nitrogen atoms can act as electron-donors toward iodine. Therefore, the lack of availability of iodine due to the formation of a CT complex, causes that iodine would no longer be accessible, and would not bind thyroglobulin. Thus, it is expected that N,N-dimethylacetamide may interact and sequester iodine acting as an anti-thyroid agent.

To study CT-complex formation, two sets of experiments were performed. The stoichiometry of the CT-complexes was established by the molar ratio method in a concentration range of DMA from 4×10^{-5} M to 1×10^{-3} M, while iodine concentration was kept constant at 4×10^{-4} M. Carbon tetrachloride was used as a solvent. The results are shown in Figure 2A, which suggests that a 1:1 [DMA]:[I_2] CT-complex was formed. A higher DMA concentration range which varied from 0.1 M to 0.3 M was used for a better observation of the CT-complex formation (Figure 2B). Upon the addition of the DMA to a solution containing I_2 (4×10^{-4} M, in CCl_4), the intense pink solution of iodine fast changed to form a stable yellow solution. Figure 2(B) shows the electronic spectrum recorded at the 360-650 nm region of the reactants. DMA reactant does not show any absorption in the region of study while iodine showed the typical electronic band located at 515 nm. As it was observed, the sequential addition of higher concentrations of DMA provoked the disappearance of the iodine band and the shift of the band to shorter wavelengths (making evident the charge transfer complex formation).

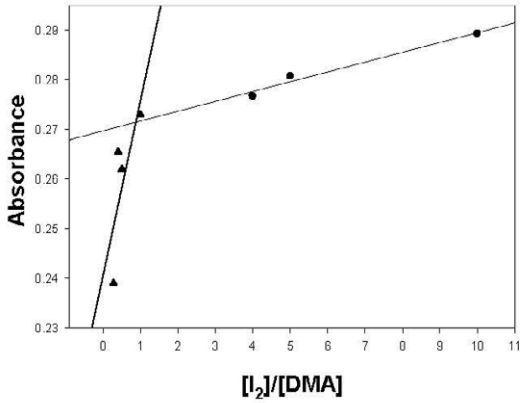


Figure 2. **(A)** *Molar ratio curve. Absorbance vs [I_2]/[DMA] ([I_2]= 4×10^{-4} M,[DMA]= 4×10^{-5}M – 1×10^{-3}M)*

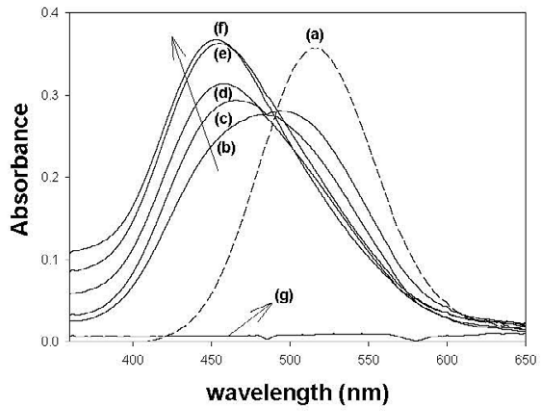


Figure 2 **(B)** *Electronic absorption spectra of (a) [I_2]= 4×10^{-4} M, (b) [I_2]= 4×10^{-4} M/[DMA] = 0.1M; (c) [I_2] = 4×10^{-4} M/[DMA] = 0.15 M, (d) [I_2] = 4×10^{-4} M/[DMA] = 0.2 M, (e) [I_2]= 4×10^{-4} M/[DMA] = 0.25 M, (f) [I_2] = 4×10^{-4} M/[DMA] = 0.3 M and (g) [DMA] = 0.3 M. Solvent = CCl_4.*

The intensity of this band increases with DMA concentration, and an isosbestic point between complexed and molecular iodine bands was observed, indicating that, under the experimental conditions, a single complex in equilibrium with DMA is present. The characteristic blue shift of the free iodine band upon complexation could be attributed to a perturbation of the iodine molecular orbital (s*) due to repulsive interactions on the iodine molecule when the CT complex is formed.[22]

To get a deeper insight into this process, the formation constant (K_{CT}) value and the molar extinction coefficient (ε) of the CT complex were determined using the

modified Benesi-Hildebrand equation for the 1:1 molar ratio. The spectroscopic determinations of these parameters were usually carried out in conditions in which the donor concentrations are higher than the acceptor and in that case the following equation can be applied:

$$\frac{[D_o] \times [A_o] \times l}{A} = \frac{1}{K_{CT}} + \frac{[D_o]+[A_o]}{\varepsilon} \quad (1)$$

where A_o and D_o are the initial concentrations of acceptors and donors, respectively, A is the absorbance at the mentioned CT bands and *l* is the cell path length (1 cm). When the ($[D_0]$ x $[A_0]$ x *l*)/A values for the 1:1 charge transfer complex are plotted against the corresponding ($[D_0]$ + $[A_0]$), a straight line is obtained with a slope of 1/ε and intercept of $1/K_{CT}$ (Figure 2C). The data obtained from this calculation ($\lambda_{max=}$ 453 nm) for the formation constant and the molar extinction coefficient were: K_{CT}=12479 M^{-1} and ε= 1223.4 $M^{-1}cm^{-1}$, respectively. The calculated values of the energy of the charge-transfer complex (E_{CT} = 2.75 eV) and the standard free energy change of complexation (ΔG^o = -23.37 KJ.mol^{-1}) suggested a spontaneous nature for the CT formation.

Considering that the magnitude of K_{CT} is an indication of the anti-thyroid activity, it can be suggested that DMA with a K_{CT} constant higher than 100 M^{-1} can be considered as a potential anti-thyroid drug.[20,21] The negative standard free energy value is an indication of the spontaneity of the interaction between DMA and iodine.

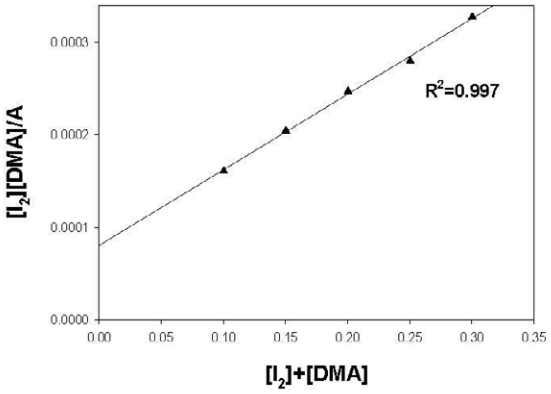
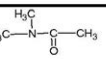
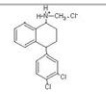
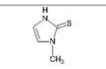
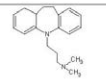
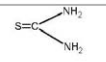
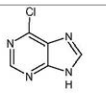
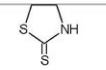
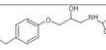


Figure 2 (C) *The plot of ([D_0]x[A_0]xl)/A vs([D_0]+[A_0])/ ε of the charge transfer complex of DMA-I_2 at 453 nm.*

Previous information reports formation constants (K_{CT}) of the binding of different types of substances with iodine. Some examples are shown in Table 1 including their chemical structures. It was not possible to perform a structure-activity relationship between DMA and any of them. Nevertheless, it is possible to make some comments. The compounds that have only one NH chemical group or one N atom in their structure have lower K_{CT} values (sertraline, imipramine, and 6-chloroquine) while those which contain N and C=S or C=O functional groups exhibited higher K_{CT} values. This behavior could probably be associated with the higher availability of these C=S[20] or C=O groups to interact with iodine and form charge-transfer complexes. Evidence of the significance of the presence of the C=O group in the formation of a charge-transfer complex was obtained in the studies of Eldaroti et. *al.*[22] They analyzed the interaction of quinidine (Qui) with quinol (QL), picric acid (PA), and dichlorodicyanobenzoquinone (DDQ) and found that the greater K_{CT} corresponded to DDQ which has two carbonyl groups in its chemical structure. But, to prove this proposal, more experiments need to be performed to get more information and to make suitable comparisons.

Table 1. *Formation constants (K_{CT}) in M^{-1} for a set of substances of pharmacological interest.*

CT-complex (1:1)	K_{CT} (M^{-1})	Structure
DMA	12479.0 (CCl_4, 25°C)	
Sertraline[15]	3277.48 ($C_2H_4Cl_2$, 25°C)	
Methimazole[16,18]	23194.0 (CCl_4, 20°C)	
Imipramine[16,18]	4907.0 (CCl_4, 20°C)	
Thiourea[18]	8825 (CCl_4, 20°C)	
6-Chloropurine[20]	7652 (DMSO, 20°C)	
Thiazolidine-2-thione[21]	1049.4 ($CHCl_3$, 25°C)	
Atenolol[25]	6300 (CH_2Cl_2, 25°C)	

Additional information can be extracted from FTIR spectral measurements. Using the same experimental conditions for K_{CT} determinations, solution FTIR spectra were measured and analyzed. Figure 2D showed three representative concentrations in different spectral ranges in which the main modifications can be observed. The FTIR spectrum of N, N-dimethylacetamide has been well studied previously.[24] The characteristic DMA band located at 1652 cm^{-1} has contributions of C=O and C-N stretching vibrations. By the addition of iodine the interaction with DMA produces a split in this spectral band (Figure 2D), appearing at 1660 cm^{-1} with a shoulder at 1650 cm^{-1}. These modifications, together with the intensification of the band at 1354 cm^{-1} ($\delta(CH_3)C$), suggest that the interaction occurs *via* the carbonyl moiety of DMA. This result would encourage the idea of the relevance of the presence of the C=O group to

obtain a stronger interaction with iodine in the charge-transfer complex.

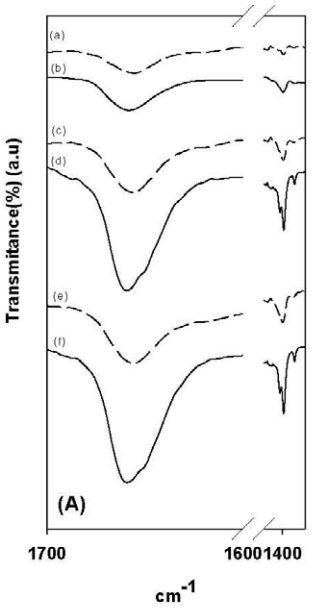


Figure 2 (D) *Infrared solution spectra of DMA-I_2(CCl_4) system: (a) [DMA] = 0.15 M, (b) [I_2] = 4 x 10^{-4} M: [DMA] = 0.15 M; (c) [DMA] = 0.25 M, (d) [I_2] = 4 x 10^{-4} M: [DMA] = 0.25 M;(e) [DMA] = 0.3 M, (f) [I_2] = 4 x 10^{-4} M: [DMA] = 0.3M.*

CONCLUSIONS

In this short communication, we were encouraged to show how a simple substance as DMA could demonstrate an additional pharmacological ability. In this sense, a short review of the literature concerning its biological activity was presented. Then, the capacity of DMA to form a charge-transfer complex with iodine was demonstrated by UV-vis spectroscopy using the Benesi-Hildebrand method to calculate the K_{CT} (12479 M^{-1}). This value is an indication of the anti-thyroid activity of DMA generated by the blockage that occurs over iodine so that it is not able to access TPO, as it has been previously postulated. Besides, a comparison with other substances was presented. The aim of these comparisons was to perform a relation between their structure and the strength of the charge-transfer complexes. The conclusion was suggested to be that the presence of C=S or C=O functional groups on the different compounds would be relevant for the strongest K_{CT} constant values obtained.

ACKNOWLEDGMENTS

The authors want to thank to the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET-PIP1999), Universidad Nacional de La Plata (UNLP 2022(SN)) and Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT-PICT-2019-00945) of Argentina for the financial support to carry out the research work. NM appreciates the scholarship received by CONICET. The authors are grateful for the academic support and the availability of the work facilities and the use of the research equipment. NM is a fellowship holder from CONICET. EGF is a research fellow of CONICET. PAMW is a research fellow of CICPBA, Argentina.

REFERENCES

1. Jouyban A., Handbook of Solubility Data for Pharmaceuticals, CRC Press, BocaRaton, FL, 2010.
2. Kirilov P., Le C.A.K., Rabehi H., Rum S., Villa C., Haftek M., Pirot F. Organogels for cosmetic and dermo-cosmetic applications–classification, preparation and characterization of organogel formulations-PART 1. *H&PC*, **2015,** *10*, 15-19.
3. Ionescu D., Blumbergs P., Silvey G.L., Pharmaceutical compositions comprising crystal forms of 5-azacytidine. US8211862B2. https://patents.google.com/patent/US8211862B2.
4. Vartak R., Menon S., Patki M., Billack B., Patel K. Ebselen nanoemulgel for the treatment of topical fungal infection. *Eur J Pharm Sci*, 2020, *148*, 105323. https://doi.org/10.1016/j.ejps.2020.105323.
5. Stephenson M. Antimicrobial sutures. US4024871A. https://patents.google.com/patent/US4024871A.
6. Armstrong W.W. Tetracycline antibiotic compositions. US4126680A. https://patents.google.com/patent/US4126680A.
7. Evers F., Mallwitz H., Wessel R., Willers C.. Topical pharmaceutical compositions. US9907807B2. https://patents.google.com/patent/US9907807B2.
8. Bull W.E., Madan S.K., Willis J.E. (1963) Amides as ligands I. Metallic complexes of N,N-Dimethylacetamide. *Inorg. Chem.* **1963**, *2*, 303-306. https://doi.org/10.1021/ic50006a016.
9. Glenn E.M. Topical pharmaceutical formulations containing N,N-dimethylacetamide as an anti-inflammatory ingredient. US3068145A. https://patents.google.com/patent/US3068145A.
10. Gorasiya S., Mushi J., Pekson R., Yoganathan S., Reznik S.E. Repurposing N,N-Dimethylacetamide (DMA), a Pharmaceutical Excipient, as a Prototype Novel Anti-inflammatory Agent for the Prevention and/or Treatment of Preterm Birth. *Curr. Pharm. Design,* 2018, *24*, 989-992. https://doi.org/10.2174/1381612824666180130121706.
11. Reznik S.E. Administration of n,n-dimethylacetamide and its monomethylated metabolites for the treatment of inflammatory disorders. US20140005272A1. https://patents.google.com/patent/US20140005272.
12. Ghayor C., Gjoksi B., Dong J., Siegenthaler B., Caflisch A., Weber F.E. N,N Dimethylacetamide a drug excipient that acts as bromodomain ligand for osteoporosis treatment. *Sci. Rep.* **2017,** *7*, 42108. https://doi.org/10.1038/srep42108.
13. Siegenthaler B., Ghayor C., Ruangsawasdi N., Weber F.E. The Release of the Bromodomain Ligand N,N-Dimethylacetamide Adds Bioactivity to a

Resorbable Guided Bone Regeneration Membrane in a Rabbit Calvarial Defect Model. *Materials,* **2020,** *13,* 501. https://doi.org/10.3390/ma13030501.
14. Bhattacharya I., Ghayor C., Pérez Dominguez A., Weber F.E. N,N-Dimethlyacetamide Prevents the High-Fat Diet-Induced Increase in Body Weight. *Front. Pharmacol.* 2019, *10*,1274. https://doi.org/10.3389/fphar.2019.01274.
15. Escudero G.E. Ferraresi Curotto V., Laino C.H., Pis Diez R., Williams P.A.M., Ferrer E.G. (2013) Sertraline and its iodine product: experimental and theoretical vibrational studies. Potential in vitro anti-thyroid activity of sertraline and iodine product toxicity with respect to male Wistar rats. *Spectrochim. Acta Part A: Molecular and Biomolecular Spectroscopy,* **2013,** *104*, **271-279.** https://doi.org/10.1016/j.saa.2012.11.076.
16. Martini N., Parente J.E., Toledo M.E., Escudero G.E., Laino C.H., Martínez Medina J.J., Echeverría G.A., Piro O.E., Lezama L., Williams P.A.M., Ferrer E.G. Evidence of promising biological-pharmacological activities of the sertraline-based copper complex: $(SerH_2)_2[CuCl_4]$. *J. Inorg. Biochem.* **2017**, *174*, 76-89. https://doi.org/10.1016/j.jinorgbio.2017.05.012.
17. Rousseau A., Comby F., Buxeraud J., Raby C. Spectroscopic Analysis of Charge Transfer Formation and Peroxidase inhibition with Tricyclic Antidepressant Drugs: Potential Anti-thyroid Action. *Biol. Pharm. Bull.*, **1996,** *19*, 726-728. https://doi.org/10.1248/bpb.19.726.
18. Raby C., Lagorce J.F., Jambut-Absil A.C., Buxeraud J., Catanzano G. The mechanism of action of synthetic antithyroid drugs: iodine complexation during oxidation of iodide. *Endocrinology*, 1990, *126,* 1683-1691. https://doi.org/10.1210/endo-126-3-1683.
19. Isaia F., Aragoni M.C., Arca M., Demartin F., Devillanova F.A., Floris G., Garau A., Hursthouse M.B., Lippolis V., Medda R., Oppo F., Pira M., Verani G. (2008) Interaction of methimazole with I_2: X-ray crystal structure of the charge transfer complex methimazole-I_2. implications for the mechanism of action of methimazole-based antithyroid drugs. *J. Med. Chem.* 2008, *51*, 4050-4053. https://doi.org/10.1021/jm8001857.
20. Fatima I., Munawar M.A., Khan M.A., Tasneem A.A., Khalil M. Antithyroid Activity of 6-Chloropurine. *J. Braz. Chem. Soc.* 2010, *2*1, 1699-1703. https://doi.org/10.1590/S0103-50532010000900015.
21. Rabie U.M., Abou-El-Wafa M.H.M., Nassar H. In vitro simulation of the chemical scenario of the action of an anti-thyroid drug: Charge transfer interaction of thiazolidine-2-thione with iodine, *Spectrochim. Acta Part A: Molecular and Biomolecular Spectroscopy*, **2011,** *78*, 512-517. https://doi.org/10.1016/j.saa.2010.11.019.
22. Eldaroti H.H., Gadir S.A., Refat M.S., Adam A.M.A. Charge-transfer interaction of drug quinidine with quinol, picric acid and DDQ: Spectroscopic characterization and biological activity studies towards understanding the drug-receptor mechanism. *J. Pharm. Anal.* **2014,** *4*, 81-95. http://dx.doi.org/10.1016/j.jpha.2013.06.003.
23. Refat M.S., Saad H.A., Adam A.M.A. (2015) Spectral, thermal and kinetic studies of charge-transfer complexes formed between the highly effective antibiotic drug metronidazole and two types of acceptors: r- and p-acceptors. *Spectrochim. Acta Part A: Molecular and Biomolecular Spectroscopy* **2015,** *141*, 202-210. http://dx.doi.org/10.1016/j.saa.2015.01.029.
24. Durgaprasad G., Sathyanarayana D.N., Patel C.C. Normal vibrations of N,N-dimethylacetamide. *Spectrochim. Acta Part A: Molecular Spectroscopy* **1972,** *28*, 2311-1318. https://doi.org/10.1016/0584-8539(72)80211-3.
25. Salem H. Spectrophotometric determination of β-adrenergic blocking agents in pharmaceutical formulations. *J. Pharm. Biomed. Anal***. 2002,** *29*, 527-538. https://doi.org/10.1016/S0731-7085(02)00100-0.