Research Article
Volume 12:7, 2022

Medicinal Chemistry

ISSN: 2161-0444 Open Access

Fabrication and Characterization of Thiolated Chitosan Microneedle Patch of Azathioprine for Transdermal Drug Delivery

Fazal Ur Rehman[1*], Riffat Latif[2], Muhammad Tahir[3] and Yasmeen Rasheed[4]

[1]*Department of Chemistry, Lahore Garrison University, Lahore, Pakistan*

[2]*Department of Pharmacy, Riphah International University, Lahore, Pakistan*

[3]*Department of Pharmacology, Mekran Medical College, Turbat-Balochistan, Pakistan*

[4]*Department of Pharmacy, Lahore College for Women University, Lahore, Pakistan*

Abstract

Micro needle patches are the novel dosage form in transdermal drug delivery systems with improved penetration to skin. In this study we fabricated micro needle patches containing azathioprine an immunosuppressant drug by using polymer thiolated chitosan that in turn was developed by adding thiol group to chitosan. These micro needle patches were further evaluated for their mechanical, physical and chemical parameters. The optimized micro needle patch having 225 needles with 665 µm length, 90 µm width showed good penetration *i.e* 84% and better percent elongation. *In-vitro* and *ex-vivo* penetration studies on rat skin using Franz diffusion cell showed sustained release of (85%) azathioprine in its comparative study with azathioprine ointment. HPLC analysis also showed 91.7% release of drug from optimized formulation of micro needle patch. Finally the recent progress proved that micro needle patches of thiolated chitosan loaded with azathioprine for transdermal drug delivery have the potential to show therapeutic outcomes with improved bioavailability and sustained release of drug over a longer period.

Keywords: Micro needle patch • Azathioprine • Thiolated chitosan • Skin penetration

Introduction

Transdermal drug delivery system is the approach by which a therapeutically effective dose of drug reaches the systemic circulation through skin penetration [1]. This delivery system is of prime attention because of increased bioavailability, penetration, ease of self-administration *i.e* noninvasive delivery of therapeutic agent, no hepatic first-pass metabolism and better patient compliance [2]. Various transdermal delivery systems have been investigated for improving drug penetration through the skin including nano carrier loaded creams, transdermal patches and micro needle patches [3]. Among these micro needles are of prime importance because of their succesfull and pain free delivery of therapeutic agent in blood stream and finally the target site [4]. Different types of micro needles are solid micro needles, coated, dissolving, and hollow and hydrogel micro needles [5]. Micro needle patches are easy to design by using moulds with length range of 150-1500 µm, width of 50-250 µm and tip of range 1-25 µm. They penetrate 50-200 µm deep in epidermis and release drug over there [6].

Thiolated Chitosan (TCS) is among the most essential and explored derivative, achieved through the attachment of the thiol group to the primary amine of chitosan *via* an amide linkage [7].

Drug profile and pharmacokinetics

Azathioprine is a potent immunosuppressant drug that has successfully reduced the rejection rates in organ transplants and autoimmune disorders. It belongs to BCS class II drugs showing low aqueous solubility (0.00402 mg/mL) and high logp=3.19 value [8]. Pharmacokinetic profile of Azathioprine varies from patient to patient that can be explained by two compartment model. After oral administration Azathioprine shows poor ADME with delayed C_{max}. Oral bioavailability is also very poor which requires 3-4 times more in oral form than IV. It also undergoes extensive distribution and metabolize completely in liver [9,10]. All the currently available dosage forms of Azathioprine have some limitations that could be resolved by introducing it as micro needle patches.

***Address to Correspondence:** Fazal Ur Rehman, Department of Chemistry, Lahore Garrison University, Lahore, Pakistan, Tel: 3467088517; E-mail: chemistryresearch5@gmail.com*

Received: 06 April, 2022, Manuscript No. AHBS-21-49208; **Editor assigned:** 08 April, 2022, PreQC No. AHBS-21-49208 (PQ); **Reviewed:** 22 April, 2022, QC No. AHBS-21-49208; **Revised:** 06 June, 2022, Manuscript No. AHBS-21-49208 (R); **Published**: 14 June, 2022, DOI: 10.37421/2161-0444.22.12.631

Problem statement

The already available conventional dosage forms of azathioprine such as ointments and tablets are to be provided repeatedly still give poor bioavailability and penetration of drug. There are problems associated with parenteral dosage forms like precipitation in aqueous media and patient education is mandatory for inhalation therapy. Micro needle patches are considered as patient friendly alternatives of conventional dosage forms and thus gained tremendous attention in the recent years. Micro needle patches are regarded as a customized or personalized dosage form for patients like pediatrics, geriatrics, bed-ridden and patient with different diseases. Going through literature, we found limited information for evaluation of azathioprine in the form of micro needle patches. Therefore, to address the issues posed by the conventional dosage forms of azathioprine, we made an attempt to develop micro needle patches of azathioprine with polymer thiolated chitosan and evaluated their physicochemical and mechanical characteristics.

Materials and Methods

Materials

Chitosan (LMW with degree of deacetylation 75–85%) (BDH, England), Thioglycolic Acid (TGA) 99%, 5,5-dithiobis (2-nitrobenzoic acid) or Ellman's reagent, 1-ethyl-3-(3- Dimethylaminopropyl) Carbodiimide (EDAC), hydroxyl- amine, sodium hydroxide, disodium di hydrogen phosphate, sodium dihydrogen phosphate, and dialysis membrane (MW 12–14 kD) were purchased from Sigma-Aldrich, Germany. PDMS moulds were imported from micro point Technology, Singapore, acetic acid (Riedel-de Haën, Germany), acetonitrile (Merck, Germany), ethanol (BDH, England). Azathioprine was received as a gift from Vaylor Pharmaceutical Industry, Pakistan.

Methods

Synthesis of thiolated chitosan: To synthesize thiolated chitosan 1 gm chitosan was dissolved in 100 ml 0.5% acetic acid solution, and then 1 gm TGA was added in it on stirring. After that EDAC was dissolved in it and and pH was adjusted to 5 using HCl 1 M solution. Continue stirring for 3 hrs, finally the mixture was dialyzed for three days at 10°C. The purified thiolated chitosan was lyophilized and stored at 4°C [11].

Fabrication of micro needle patch: Three formulations containing mixture of 1%, 2% and 3% thiolated chitosan solution and Azathioprine (3 mg dissolved in methanol) were prepared and loaded on micromoles (Micro point Technology, Singapore) using casting method to develop micro needle patches. Afterward, filled mould was dried at room temperature for 24 hrs and dried patches were removed from mould by using adhesive backing tape.

Characterization of micro needle patch

SEM studies: Differential scanning was performed by using Scanning Electron Microscope (SEM) in which the dimensional analysis of micro needle patches was performed *i.e* height of needle, length and width of patch and needle pitch etc [12]. In this procedure the micro needle patch was attached on copper grid that was coated with carbon and a drop of ammonium molbydate solution was poured on it [13].

FTIR analysis: Fourier transform infrared studies were performed using FTIR (Agilant technology) to check the compatibility studies of thiolated chitosan, Azathioprine and their micro needle patch [14]. In this study spectras of all the ingredients and in patch form were studied and compared.

Moisture contents: The moisture contents of micro needle patches were determined by using moisture analyser (Sartorious, Germany) by following the reported method [15].

Thickness: The thickness of patches was observed by using vernier caliper. The average thickness of patch should be between 50-250 µm. Patch thickness was observed from all the four sides and from center and average of all should be mentioned [16].

Tensile strength: Tensile strength of micro needle patches was observed by using auto tensile tester (Shimatzu). The patch was hold in the jaws of tensile tester and force was applied until patch torn in to two pieces. The displayed force on tester would be noted as a result [17].

The formula to calculate tensile strength is as below:

Tensile strength=Load at failure x 100 /Patch thickness x Patch width

Percent elongation: Percent elongation of patch was also measured by tensile tester. Patch was placed in the jaws of tester and force was applied. The increase in length of patch after applying load was noted and results were calculated by comparing it with initial length [18,19].

Formula for result calculation is:

Percent Elongation=Final Length of Patch/Initial length x 100

Penetration study: Skin penetration study of micro needle patch was performed by using para film by following reported method [20]. The Para film was folded into eight folds. The para film was placed on smooth surface and micro needle patch was placed over it, then force was applied manually for 30 seconds. Rhodamine B was used over the surface; it will help to clarify the holes formed by the needles of patch and also any needle damaged on patch after penetration [21]. Finally the patch was removed and both patch and the film were observed microscopically for piercing and any damaged tip of needles [22].

The percentage compression was calculated using formula:

%compression=Hbc-Hac/Hbc x 100.

***In-Vitro* drug release study:** *In-vitro* drug release study was performed by using Franz Diffusion cell. The micro needle patch with para film was placed between sealed chambers of the cell (donor and recipient) [23,24]. The compartment was filled with buffer solution with pH 7.4, sample was drawn at predetermined intervals for 48 hrs and analyzed by HPLC method [25]. Comparative study was performed by azathioprine ointment as control.

HPLC method validation: Quantitative study was performed using HPLC (Shimadzu). The reported method was first validated using linear equation. The C-18 column (5 µ × 4.6 mm × 150 mm) was used for the analysis, and the mobile phase comprised of Acetonitrile (ACN)/Trifluoroacetic Acid (TFA) (70:30) with a flow rate 1.2 mL/min. The run time was set at 10 min with injection volume 20

µL. The absorbance was measured at 214 nm using UV-visible detector [26].

Results and Discussion

SEM studies

Scanning electron microscopic studies were applied to see the morphology of three microneedle patches and patch 2 showed the intact needles of length 665 µm with sharp pointed end (Figure 1a-e).

FTIR analysis

FTIR studies were performed to check the compatibility studies of thiolated chitosan, Azathioprine and their micro needle patch (Figure 2). The spectra of chitosan, thiolated chitosan showed clear peaks at 3352 cm^{-1} and 3210 cm^{-1} indicating O-H and N-H functional groups. Furthermore, the peak near 1610 cm^{-1} is decreased indicating that N-H group is partially conjugated to TGA resulting in successful synthesis of thiolated chitosan. Moreover, the peaks in spectra of Azathioprine were also observed in the spectra of patch indicating the compatibility of thiolated chitosan and Azathioprine.

Moisture contents

The moisture contents of micro needle patches were found between 3.0 to 3.2% (Figure 3a).

Thickness

The thickness of patches was observed between 0.043 mm to 0.04 mm. The results showed non-significant variation in thickness indicating homogenecity in mould filling and mould fabrication (Figure 3b).

Tensile strength

Micro needle patch 2 showed better tensile strength *i.e* 0.05 mPa. Tensile strength of patch 1 and 3 was 0.044 mPa and 0.048 mPa respectively (Figure 3c).

Percent elongation

This character was analyzed by using tensile tester. Results showed the maximum elongation of 35% by formulation no. 2. Patch 1 and 3 showed elongation of 30% and 33% respectively (Figure 3d).

Penetration study

Skin penetration study of micro needle patch was performed by using para film to confirm the portion made by patch needles. The results were shown in Figure 4a-f. It was observed that the micro needle patches have penetrated into para film layer upto depth of 485 µm. Also the number of holes was counted under the microscope that was 225 holes. Patch needles were also intact which indicate mechanical strength of needles.

Moreover, *ex-vivo* studies for skin penetration were conducted on mice skin in which micro needle patch showed penetration across stratum corneum and deep into dermis. Results shown in Figure 4g-i.

In-Vitro drug release study

In-vitro drug release study was performed by using Franz diffusion cell (Figure 5a,b). Test was conducted for 48 hrs and the results shown in Figure 5c in which maximum drug release was 85%. The delivery was also observed with Azathioprine ointment in which drug release was only 40% in same duration. After drug release change in patch morphology was observed under microscope (Figure 5e-g), which is the evidence of Azathioprine release in a sustained manner in 48 hrs.

HPLC method validation

HPLC chromatogram of drug in pure form and in patch was shown in Figure 6 a. Peaks were observed at retention time of 6.77 minutes. The absorbance observed by all the three formulations of patches was between 87.5% to 91.7%, out of which the maximum absorbance was shown by microneedle patch 2 *i.e* 91.7%.

Also the analytical method was validated through linearity graph at different concentrations of drug (50, 100, 150, 200 and 250 µg/mL). Linearity observed with R_2=0.9965 Figure 6b.

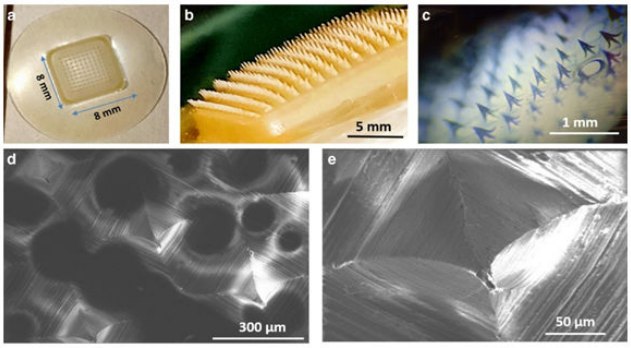

Figure 1. A) Micro needle patch with 2% thiolated chitosan; b) macroscopic view of patch showing clear sharp needles; c) microscopic view of micro needles; d) Scanning Electron Microscopic (SEM) view of micro needle patch and e) SEM of single micro needle showing pyramid shape with a sharp-pointed end.

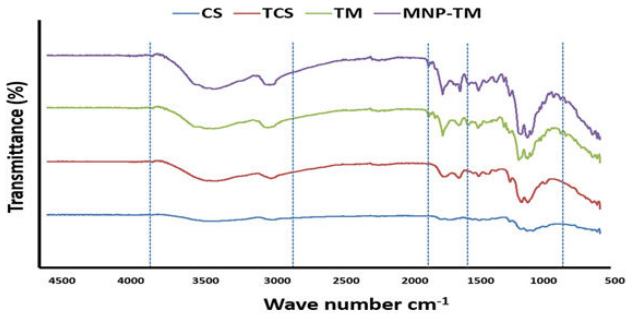

Figure 2. FTIR analysis of Chitosan (CS), Thiolated Chitosan (TCS), Azathioprine (TM), and Microneedle Patch (MNP) showing characteristic peaks of the materials over the scan 500–4500 cm^{-1}.

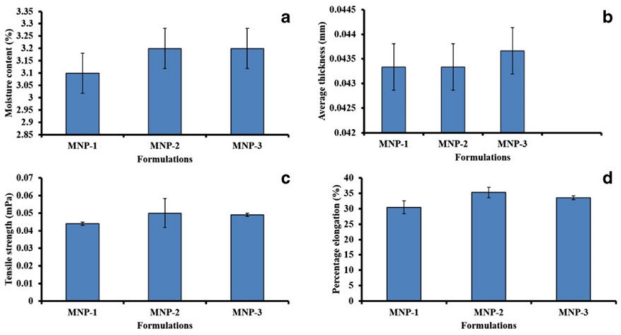

Figure 3. Evaluation of mechanical properties of the micro needle patch synthesized using different thiolated chitosan concentration. A) Moisture content estimation; b) thickness measurement; c) tensile strength measurement and d) percentage elongation.

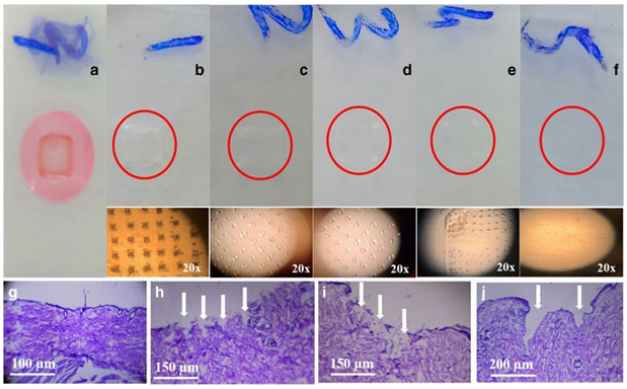

Figure 4. Insertion studies using eight layers of Para film M. a) Rhodamine B–loaded micro needle patch attached to the eight layer-folded Para film M; b–f) unfolded single para film layers showing macroscopic and microscopic view of holes created through patch insertion. Histological evaluation of mice skin after micro needle patch insertion; g) normal skin with intact layers; h-i)skin showing punctures in the skin after micro needle patch insertion and j) high magnification image of skin showing puncture.

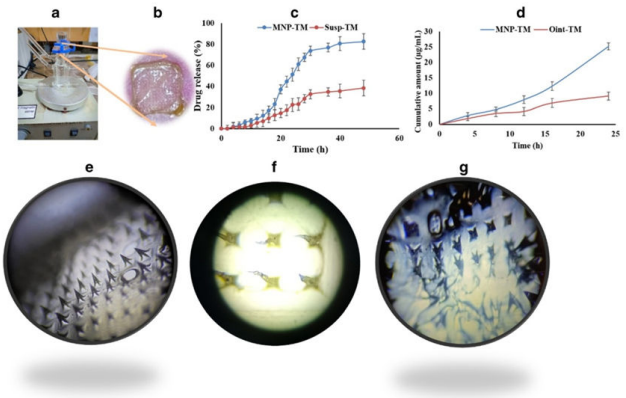

Figure 5. *Ex vivo* permeation studies through Franz diffusion cell: a Franz cell assembly, b mice skin with applied micro needle patch, and c-d Azathioprine release studies from micro needle patch and ointment for 48 h. Microscopic view of the patch e before insertion showing sharp- pointed needles, f micro needles after 24-h insertion into skin showing needles with changed morphology, and g micro needle patch after 48 h showing maximum dissolved needles.

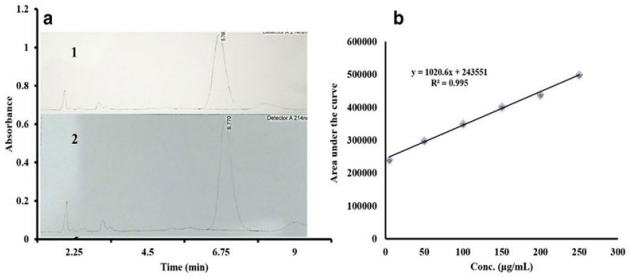

Figure 6. A HPLC chromatograph of (1) pure Azathioprine and (2) Azathioprine release from micro needle patch. B Standard curve of Azathioprine showing linearity of data over 50–250 µg/mL with $R_2$0.9956.

Conclusion

The results in this article indicates that the micro needle patch 2 fabricated with 2% thiolated chitosan showed the best results in terms of tensile strength, percent elongation, *in-vitro* and *ex-vivo* skin penetration and drug release as compared to other two formulations of patches. The micro needle patch successfully pierced the skin facilitating better control of drug release from patch over the period of 48 hrs in a sustained manner. Thus micro needle patch of azathioprine containing thiolated chitosan can be characterized for better therapeutic results as compared with already available azathioprine ointment.

References

1. Garland MJ, Caffarel–Salvador E, Migalska K and Woolfson AD, et al. Dissolving polymeric microneedle arrays for electrically assisted transdermal drug delivery. *J Control Release* 159 (2012): 52-59.
2. Das Kurmi B, Tekchandani P, Paliwal R and Rai Paliwal S, et al. Transdermal drug delivery: opportunities and challenges for controlled delivery of therapeutic agents using nanocarriers. *Curr Drug Metab* 18 (2017): 481-495.
3. Waghule T, Singhvi G, Dubey SK and Pandey MM, et al. Microneedles: A smart approach and increasing potential for transdermal drug delivery system. *Biomed Pharmacother* 109 (2019): 1249-1258.
4. Chen J, Huang W, Huang Z and Liu S, et al. Fabrication of tip-dissolving microneedles for transdermal drug delivery of meloxicam. *AAPS PharmSci Tech* 19 (2018): 1141-1151.
5. Parrilla M, Cuartero M, Padrell Sánchez S and Rajabi M, et al. Wearable all-solid-state potentiometric microneedle patch for intradermal potassium detection. *Anal Chem* 91 (2018): 1578-1586.
6. Kathuria H, Kang K, Cai J and Kang L, et al. Rapid microneedle fabrication by heating and photolithography. *Int J Pharm* 575 (2020): 118992.
7. Luo Q, Han Q, Wang Y and Zhang H, et al. The thiolated chitosan: Synthesis, gelling and antibacterial capability. *Int J Biol Macromol* 139 (2019): 521-530.
8. Gardenier JC, Kataru RP, Hespe GE and Savetsky IL, et al. Topical Azathioprine for the treatment of secondary lymphedema. *Nature communications* 8 (2017): 1-11.
9. Campagne O, Mager DE and Tornatore KM. Population pharmacokinetics of Azathioprine in transplant recipients: what did we learn about sources of interindividual variabilities? *J Clin Pharmacol* 59 (2019): 309-325.
10. Guo YM, Abumiya M, Yamashita T and Ubukawa K, et al. Pharmacokinetics of Azathioprine coadministered with letermovir in

allogeneic hematopoietic stem cell transplantation patients. *Clin Pharmacol Drug Dev* 8 (2019): 411-412.

11. Dünnhaupt S, Barthelmes J, Thurner CC and Waldner C, et al. S-protected thiolated chitosan: synthesis and *in vitro characterization. Carbohydr Polym* 90 (2012): 765-772.
12. Gholami S, Mohebi MM, Hajizadeh-Saffar E and Ghanian MH, et al. Fabrication of microporous inorganic microneedles by centrifugal casting method for transdermal extraction and delivery. *Int J Pharm* 558 (2019): 299-310.
13. Ahmed MR, Rajadas J, Sun W and Ahmed MIN, et al. United States Patent and Trademark Office. Washington, DC: U.S. 2021
14. Prasad SR, Elango K, Damayanthi D and Saranya JS, et al. Formulation and evaluation of azathioprine loaded silver nanopartilces for the treatment of rheumatoid arthritis. *AJBPS* 3 (2013): 28-32.
15. Van den Dool H, Huang J and Fan Y. Performance and analysis of the constructed analogue method applied to US soil moisture over 1981–2001. *J Geophys Res Atmos* 108 (2003).
16. Parrilla M, Cuartero M, Padrell Sánchez S and Rajabi M, et al. Wearable all-solid-state potentiometric microneedle patch for intradermal potassium detection. *Anal chem* 91 (2018): 1578-1586.
17. Yang SJ, Jeong JO, Lim YM and Park JS, et al. Synthesis and characterization of PVP microneedle patch using metal bioelectrodes for novel drug delivery system. *Mater Des* 201 (2021): 109485.
18. Khan TA, Peh KK and Ch'ng H. Mechanical, bioadhesive strength and biological evaluations of chitosan films for wound dressing. *J Pharm Pharm Sci* 3 (2000): 303–311.
19. Nguyen HX, Bozorg BD, Kim Y and Wieber A, et al. Poly (vinyl alcohol) microneedles: fabrication, character- ization, and application for transdermal drug delivery of doxorubicin. *Eur J Pharm Biopharm* 129 (2018): 88–103.
20. Lee JW, Park J-H and Prausnitz MR. Dissolving microneedles for transdermal drug delivery. *Biomaterials* 29 (2008): 2113–2124.
21. Liu S, Jin M-n, Quan YS and Kamiyama F, et al. The development and characteristics of novel microneedle arrays fabricated from hyaluronic acid, and their application in the transdermal delivery of insulin. *J Control Release* 61 (2012): 933–941.
22. Sohail MF, Shah PA, Tariq I and Saeed-ul-Hassan S, et al. Development and *in vitro* evaluation of flurbiprofen microcapsules prepared by modified solvent evaporation tech- nique. *Trop J Pharm Res* 13 (2014): 1031–1038.
23. Saboktakin MR, Tabatabaie RM, Maharramov A and Ramazanov MA, et al. Synthesis and *in vitro* evaluation of thiolated chitosan-dextran sulfate nanoparticles for the delivery of letrozole. *J Pharm Educ Res* 1 (2010): 62–67.
24. Chen M-C, Ling M-H, Lai K-Y and Pramudityo E, et al. Chitosan microneedle patches for sustained transdermal delivery of macromolecules. *Biomacromolecules* 13 (2012): 4022–4031.
25. Singh TRR, McCarron PA, Woolfson AD and Donnelly RF, et al. Physicochemical characterization of poly (ethylene glycol) plasticized poly (methyl vinyl ether-co-maleic acid) films. *J Appl Polym Sci* 112 (2009): 2792–2799.
26. Liu S, Jin M-n, Quan Y-s, Kamiyama F and Kusamori K, et al. Transdermal delivery of relatively high molecular weight drugs using novel self-dissolving microneedle arrays fabricated from hyaluronic acid and their characteristics and safety after application to the skin. *Eur J Pharm Biopharm* 86 (2014): 267–276.

How to cite this article: Rehman, Fazal Ur, Riffat Latif, Muhammad Tahir and Yasmeen Rasheed. "Fabrication and Characterization of Thiolated Chitosan Microneedle Patch of Azathioprine for Transdermal Drug Delivery." *Med Chem*12 (2022) : 631