

Synthesis and Antimicrobial Evaluation of 4*H*-Pyrans and Schiff Bases Fused 4*H*-Pyran Derivatives as Inhibitors of *Mycobacterium bovis* (BCG)

Seyedeh Mahbobeh Mahdavi^{a, b}, Azizollah Habibi^{a*}, Hadi Dolati^{a, b}, Seyed Mohammad Shahcheragh^{a, c}, Soroush Sardari^c and Parisa Azerang^c

^aDepartment of Organic Chemistry, Faculty of Chemistry, Kharazmi University, Tehran, Iran. ^bPhytochemistry Research Center, Department of Pharmacognosy, School of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran. ^cDrug Design and Bioinformatics Unit, Department of Medical Biotechnology, Biotechnology Research Center, Pasteur Institute of Iran, Tehran, Iran.

Abstract

The focus of our study is the synthesis and biological activity evaluation of a series of 4*H*-Pyran compounds and schiff bases fused 4*H*-Pyran derivatives which are known to possess a wide variety of biological activities. In this paper at first a simple and efficient one-pot synthesis of 4*H*-Pyran s from the three-component reaction between malononitrile, aldehydes, and active methylene compounds in the presence of *N*-methylmorpholine (NMM) as catalyst at room temperature is reported, the reaction between these synthesized products and trimethylorthoformate or triethylorthoformate to produce schiff base compounds were also considered. The key advantages of synthesis of 4*H*-Pyran derivatives are short reaction time, high yield, and simple work-up. Then, these compounds were evaluated for anti-*Mycobacterium* activity against *Mycobacterium bovis* (Bacillus Calmette–Guerin). The preliminary results indicated that most of the tested compounds showed relatively good activity against the test organism. Moreover, antifungal activities of these compounds were evaluated. Finally, their effect was more noticeable on *Mycobacterium bovis* (BCG).

Keywords: Antifungal; Anti-*Mycobacterium*; 4*H*-Pyran; *N*-Methylmorpholine; Schiff bases.

Introduction

Infectious diseases remain the largest cause of death in the world today, greater than cardiovascular disease or cancer (1). Based on an estimate by the World Health Organization (WHO) about two million people worldwide are infected with *Mycobacterium tuberculosis* (TB) each year and the number of new cases of tuberculosis has increased in recent years (2). Because of this, researchers showed interest in

discovering new leads and active compounds effective against *Mycobacterium* species (3, 4).

Multicomponent reactions (MCRs) are special types of synthetically useful organic reactions in which three or more different starting materials react to give a final product in a one-pot protocol. Most of these reactions are atom-efficient processes by incorporating the essential parts of the starting materials into the final product. Major applications of MCRs described until today arise from the area of drug discovery (5, 6).

4*H*-Pyran s belong to an important class of heterocyclic compounds due to their wide

* Corresponding author:
E-mail: habibi@khu.ac.ir

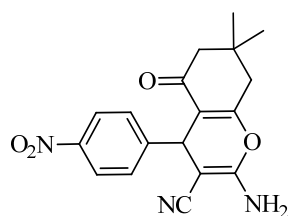


Figure 1. The structure of 2-amino-4H-Pyran compound with antibacterial activity.

biological and pharmaceutical properties, such as diuretic, spasmolytic, anticancer, anti-coagulant (7), antimicrobial (8), mutagenicity (9), antitumor (10), antiviral (11, 12), sex pheromone (13), anti-proliferative (14). Furthermore, these compounds can be used for the treatment of schizophrenia, alzheimer's disease and mycolonous diseases (15). In addition, 4H-Pyran is a constituent of some natural products (16, 17) and a number of 2-amino-4H-Pyrans are useful as photoactive materials (18). Also, the antifungal and anti-TB activities of some 4H-Pyrans with different substitutions as a novel pharmacophore have also been investigated (19, 20).

On the other hand, schiff bases are compounds containing C=N group and interpret an important category of organic compounds, particularly in the medical and pharmaceutical fields due to a wide spectrum of biological activities like fungicidal, anticancer, antibacterial, herbicidal activities (21) antiviral (22), anti-inflammatory (23), anti-HIV (24) and antioxidant property (25). Additionally, schiff bases containing heterocycles possess excellent biological activities such as fungicidal, anticancer, antiviral, anti-HIV and bactericidal (26).

The antibacterial properties of some compounds derived from 2-amino-4H-Pyran (Figure 1) (9) prompted us to undertake the synthesis of various novel schiff bases derived from 4H-Pyran derivatives with the aim of improving activity.

The main purpose of this project is to develop synthetic methods in heterocyclic chemistry via Multicomponent Reactions (MCRs); specifically in schiff bases fused 4H-Pyran derivatives and especially to evaluate their anti-TB and antifungal activities.

Experimental

General

Melting points were measured using a capillary tube method with a Barnstead Electrothermal 9200 apparatus. FTIR spectra were recorded using KBr disks on Perkin-Elmer Spectrum RXI FT-IR Spectrophotometer. ¹H and ¹³C NMR spectra were recorded on a Bruker AMX spectrometer at 300 MHz. The elemental analysis was performed with an Elementar Analysensystem GmbH Perkin Elmer.

General procedure for the preparation of 2-amino-4H-Pyran derivatives

NMM (30 mol%) was added to a mixture of aldehydes (1 mmol), malononitrile (1 mmol) and 1, 3-diketone compounds (1 mmol) in ethanol (5 mL). The reaction was stirred at room temperature. After completion of the reaction, as indicated by TLC, the solid product was collected by filtration and purified by washing with water.

General procedure for the preparation of Schiff base derivatives

In a 10 mL round bottom flask, a mixture of 2-amino-4H-Pyran (1 mmol) and (trimethylorthoformate or triethylorthoformate) (2 mL) were placed over a magnetic stirrer and the contents were stirred and then 5 drops of acetic anhydride was added to this stirred mixture. The reaction mixture was heated under reflux for 7-9 h. The progress of the reaction was monitored by TLC. After completion of the reaction, the reaction mixture was evaporated under reduced pressure. The residue was dissolved in ethanol and water was added to the mixture and was stirred. The solid was collected by filtration, washed with water to obtain pure products.

In-vitro evaluation of anti-mycobacterial activity

The broth microtiter dilution method against BCG (1173P2) has been used for *in-vitro* anti-mycobacterial activity evaluations of the compounds. Ethambutol and isoniazid were used as standard controls.

At first the test compounds were dissolved in DMSO to give a concentration of 1 or 2 mg/mL.

All the wells of micro plates received 100 μ L of freshly prepared Middle Broke 7H9 medium (Himedia, India), except for the first column. The medium in the test wells during incubation, 100 μ L was added to the first column of 96-well plates. Then 100 μ L of test compounds with desired concentrations (1000 or 2000 μ g/mL) were added to the wells of the first row and serial double dilution was made from the first row to the last (each concentration was assayed in duplicate). Microbial suspension equal to 100 μ L of BCG (1173P2), which had been prepared with standard concentration of 0.5 Mcfarland and diluted with 1:10 proportion by the distilled water, was added to all test wells. Incubation has been done for 96 h at 37 °C. Then, 12 μ L Tween 80 10% and 20 μ L Alamar blue 0.01% (Himedia, India) were added to each test well. The results were assessed after 24 and 48 h. The change to pink color against blue color is interpreted as a sign of bacterial growth. The MIC (minimum inhibitory concentration) is defined as the lowest drug concentration inhibiting color change from blue to pink. Ethambutol and isoniazid (Iranbaru, Tehran) were used as positive and DMSO as negative control (27).

Antifungal screening

Candida albicans ATCC 10231 was used as test strain. The compounds were dissolved in dimethyl sulfoxide (DMSO) to reach concentration of 10 mg/mL. Antifungal activity tests performed by broth microdilution method. The absorbance was read at 530 nm in order to yield the desired transmittance of 75 to 77%. Working fungal culture was prepared from the stock fungal culture, a 1:1000 dilution with broth (e.g. 10 μ L stock fungal culture: 10 μ L broth) was prepared. Sabouraud maltose broth (DIFCO, Becton, Dickinson, USA) was used as the growth medium. Modified antimicrobial susceptibility testing is based on NCCLS M27-A method (28). Firstly, 100 μ L of the broth was added to all wells of microplate, after that 40 μ L of the test compounds and again 60 μ L broth were added to well A. Secondly, a 100 μ L solution serially diluted from well A by taking 100 μ L into well B was obtained. This two-fold dilution was continued down the plate and 100 μ L from the last well (H) was discarded. Finally,

all the wells were filled with 100 μ L of working fungal culture. Ketoconazole and amphotericin B (AmB) were used as a reference in the antifungal test. Wells containing serial dilution of DMSO and broth were prepared as control tests. The plate was sealed and incubated at 37 °C for 24 to 48 h. The minimum inhibitory concentration (MIC) values were obtained by reading the lowest concentration of compound in the well showing no growth.

5-acetyl-2-amino-4-(3-methoxyphenyl)-6-methyl-4H-Pyran -3-carbonitrile (compound 4h)

Yellow powder, mp: 157-160 °C. IR (KBr) (λ_{\max} , cm^{-1}): 3334, 3185, 2193, 1676. ^1H NMR (DMSO- d_6 , 300 MHz): δ 2.05 (s, 3H, CH₃), 2.22 (s, 3H, CH₃), 3.71 (s, 3H, CH₃), 4.43 (s, 1H, CH), 6.69-6.82 (m, 3H, ArH), 6.87 (s, 2H, NH₂), 7.22-7.27 (m, 1H, ArH). ^{13}C NMR (DMSO- d_6 , 75.0 MHz): δ 18.4, 29.8, 55.0, 57.6, 111.7, 113.3, 114.8, 119.3, 119.8, 129.9, 146.2, 154.8, 158.3, 159.4, 198.4. Anal. calcd. for C₁₆H₁₆N₂O₃ (284.31): C, 67.59; H, 5.67; N, 9.85. Found: C, 67.44; H, 5.75; N, 9.77.

5-acetyl-2-amino-4-(2-chlorophenyl)-6-methyl-4H-Pyran -3-carbonitrile (compound 4i)

Cream powder, mp: 130-132 °C. IR (KBr) (λ_{\max} , cm^{-1}): 3320, 3198, 2195, 1688, 1658. ^1H NMR (DMSO- d_6 , 300 MHz): δ 2.04 (s, 3H, CH₃), 2.24 (s, 3H, CH₃), 4.96 (s, 1H, CH), 6.93 (s, 2H, NH₂), 7.19-7.43 (m, 4H, ArH). ^{13}C NMR (DMSO- d_6 , 75.0 MHz): δ 18.6, 29.6, 44.7, 56.2, 114.2, 119.3, 128.1, 128.8, 129.6, 130.0, 131.7, 141.5, 155.7, 158.6, 197.9. Anal. calcd. for C₁₅H₁₃ClN₂O₂ (288.73): C, 62.40; H, 4.54; N, 9.70. Found: C, 62.24; H, 4.57; N, 9.65.

Methyl N-(3-cyano-7,7-dimethyl-4-(4-nitrophenyl)-5-oxo-5,6,7,8-tetrahydro-4H-chromen-2-yl) formimidate (compound 5a)

Cream powder, mp: 168-171 °C. IR (KBr) (λ_{\max} , cm^{-1}): 2963, 2212, 1661, 1612. ^1H NMR (DMSO- d_6 , 300 MHz): δ 0.97 (s, 3H), 1.04 (s, 3H), 2.20 (d, J=16.1 Hz, 1H), 2.27 (d, J=16.1 Hz, 1H), 2.59 (s, 2H), 3.85 (s, 3H), 4.65 (s, 1H), 7.56 (d, J=8.7 Hz, 2H), 8.19 (d, J=8.7 Hz, 2H), 8.57 (s, 1H). ^{13}C NMR (DMSO- d_6 , 75.0 MHz): δ 27.1,

28.1, 31.9, 36.9, 49.9, 55.0, 81.4, 110.7, 116.9, 123.8, 129.3, 146.7, 150.1, 156.4, 162.9, 163.5, 195.8. Anal. calcd. for $C_{20}H_{19}N_3O_5$ (381.38): C, 62.99; H, 5.02; N, 11.02. Found: C, 63.03; H, 4.87; N, 10.92.

Methyl N-(3-cyano-7,7-dimethyl-4-(3-nitrophenyl)-5-oxo-5,6,7,8-tetrahydro-4H-chromen-2-yl)formimidate (compound 5b)

Cream powder, mp: 152-155 °C. IR (KBr) (λ_{max} , cm^{-1}): 2967, 2207, 1670, 1619. 1H NMR (DMSO- d_6 , 300 MHz): δ 0.98 (s, 3H), 1.05 (s, 3H), 2.14 (d, J=16.1 Hz, 1H), 2.27 (d, J=16.1 Hz, 1H), 2.60 (s, 2H), 3.30 (s, 3H), 4.7 (s, 1H), 7.65 (t, J=7.8 Hz, 1H), 7.76 (d, J=7.7 Hz, 1H), 8.08-8.13 (m, 2H), 8.62 (s, 1H). ^{13}C NMR (DMSO- d_6 , 75.0 MHz): δ 26.9, 28.2, 32.0, 36.7, 49.9, 55.0, 81.6, 110.7, 116.9, 122.3, 122.4, 130.2, 134.7, 145.0, 147.9, 156.4, 162.9, 163.6, 195.8. Anal. calcd. for $C_{20}H_{19}N_3O_5$ (381.38): C, 62.99; H, 5.02; N, 11.02. Found: C, 63.10; H, 5.06; N, 10.99.

Methyl N-(3-cyano-4-(4-methoxyphenyl)7,7-dimethyl-5-oxo-5,6,7,8-tetrahydro-4H-chromen-2-yl)formimidate (compound 5c)

Cream powder, mp: 162-163 °C. IR (KBr) (λ_{max} , cm^{-1}): 1956, 2209, 1667, 1612. 1H NMR (DMSO- d_6 , 300 MHz): δ 0.96 (s, 3H), 1.03 (s, 3H), 2.12 (d, J=16.1 Hz, 1H), 2.25 (d, J=16.1 Hz, 1H), 2.56 (s, 2H), 3.71 (s, 3H), 3.83 (s, 3H), 3.84 (s, 1H), 6.86 (d, J=8.6 Hz, 2H), 7.13 (d, J=8.6, 2H), 8.57 (s, 1H). ^{13}C NMR (DMSO- d_6 , 75.0 MHz): δ 26.9, 28.3, 31.9, 36.3, 50.0, 54.8, 55.0, 82.9, 111.8, 113.9, 117.2, 128.8, 134.8, 155.6, 158.3, 162.2, 162.6, 195.7. Anal. calcd. for $C_{21}H_{22}N_2O_4$ (366.41): C, 68.84; H, 6.05; N, 7.65. Found: C, 68.98; H, 6.07; N, 7.44.

Methyl N-(3-cyano-4-(3-methoxyphenyl)7,7-dimethyl-5-oxo-5,6,7,8-tetrahydro-4H-chromen-2-yl)formimidate (compound 5d)

Cream powder, mp: 148-150 °C. IR (KBr) (λ_{max} , cm^{-1}): 2964, 2212, 1662, 1610. 1H NMR (DMSO- d_6 , 300 MHz): δ 0.98 (s, 3H), 1.02 (s, 3H), 2.14 (d, J=16.1 Hz, 1H), 2.26 (d, J=16.1 Hz, 1H), 2.58 (s, 2H), 3.72 (s, 3H), 3.84 (s, 3H), 4.37 (s, 1H), 6.77 (t, J=8.4 Hz, 1H), 6.82 (d, J=2.1 Hz, 1H), 7.21-7.27 (m, 2H), 8.57 (s, 1H). ^{13}C NMR (DMSO- d_6 , 75.0 MHz): δ 26.9, 28.2, 31.9,

37.1, 50.0, 54.9, 82.7, 111.5, 117.1, 127.2, 127.7, 128.5, 142.7, 155.8, 162.3, 162.9, 195.7. Anal. calcd. for $C_{21}H_{22}N_2O_4$ (366.41): C, 68.84; H, 6.05; N, 7.65. Found: C, 68.69; H, 6.12; N, 7.61.

Ethyl N-(4-(4-chlorophenyl)-3-cyano7,7-dimethyl-5-oxo-5,6,7,8-tetrahydro-4H-chromen-2-yl)formimidate (compound 5e)

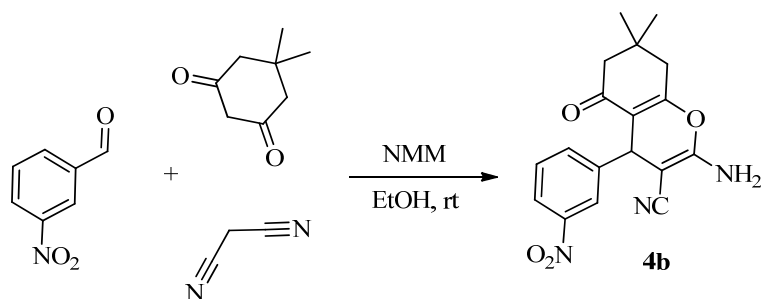
Cream powder, mp: 167-170 °C. IR (KBr) (λ_{max} , cm^{-1}): 2209, 1663, 1605. 1H NMR (DMSO- d_6 , 300 MHz): δ 1.05 (s, 3H), 1.13 (s, 3H), 1.38 (t, J=7.1 Hz, 3H), 2.39 (s, 2H), 2.49 (s, 2H), 4.40 (q, J=7.2 Hz, 3H), 4.51 (s, 1H), 7.20-7.30 (m, 4H), 8.25 (s, 1H). ^{13}C NMR (DMSO- d_6 , 75.0MHz): δ 13.8, 27.6, 28.9, 32.3, 37.1, 40.7, 50.6, 64.5, 83.5, 112.6, 116.9, 128.9, 129.3, 133.3, 140.6, 155.9, 159.3, 162.3, 195.8. Anal. calcd. for $C_{21}H_{21}ClN_2O_3$ (384.86): C, 65.54; H, 5.50; N, 7.28. Found: C, 65.38; H, 5.49; N, 7.16.

Methyl N-(4-(2-chlorophenyl)-3-cyano7,7-dimethyl-5-oxo-5,6,7,8-tetrahydro-4H-chromen-2-yl)formimidate (compound 5f)

Cream powder, mp: 155-157 °C. IR (KBr) (λ_{max} , cm^{-1}): 2954, 2210, 1664, 1618. 1H NMR (DMSO- d_6 , 300 MHz): δ 0.99 (s, 3H), 1.05 (s, 3H), 2.11 (d, J=16.1 Hz, 1H), 2.26 (d, J=16.1 Hz, 1H), 2.58 (s, 2H), 3.84 (s, 3H), 4.91 (s, 1H), 7.23-7.34 (m, 4H), 8.59 (s, 1H). ^{13}C NMR (DMSO- d_6 , 75.0 MHz): δ 27.0, 28.3, 31.9, 34.3, 40.9, 49.9, 54.9, 81.1, 110.8, 116.7, 127.7, 128.9, 129.6, 130.5, 132.4, 139.7, 156.2, 162.5, 163.6, 195.6. Anal. calcd. for $C_{20}H_{19}ClN_2O_3$ (370.83): C, 64.78; H, 5.16; N, 7.55. Found: C, 64.55; H, 5.08; N, 7.67.

Methyl N-(3-cyano-7,7-dimethyl-5-oxo-4-phenyl-5,6,7,8-tetrahydro-4H-chromen-2-yl)formimidate (compound 5g)

Cream powder, mp: 177-178 °C. IR (KBr) (λ_{max} , cm^{-1}): 2956, 2205, 1667, 1664. 1H NMR (DMSO- d_6 , 300 MHz): δ 0.97 (s, 3H), 1.04 (s, 3H), 2.13 (d, J=16.2 Hz, 1H), 2.26 (d, J=16.2 Hz, 1H), 2.58 (s, 2H), 3.84 (s, 3H), 4.40 (s, 1H), 7.21-7.34 (m, 5H), 8.58 (s, 1H). ^{13}C NMR (DMSO- d_6 , 75.0 MHz): δ 26.9, 28.2, 31.9, 37.1, 50.0, 54.9, 82.7, 111.5, 117.1, 127.2, 127.7, 128.5, 142.7, 155.8, 162.3, 162.9, 195.7. Anal. calcd. for $C_{20}H_{20}N_2O_3$ (336.38): C, 71.41; H, 5.99; N, 8.33. Found: C, 71.24; H, 6.01; N, 8.30.



Scheme 1. Synthesis of schiff base compounds.

Methyl N-(5-acetyl-3-cyano-4-(3-methoxyphenyl)-6-methyl-4H-Pyran-2-yl)formimidate (compound 5h)

Cream powder, mp: 160-162 °C. IR (KBr) (λ_{\max} , cm^{-1}): 2967, 2207, 1687. ^1H NMR (DMSO- d_6 , 300 MHz): δ 2.09 (s, 3H), 2.29 (s, 3H), 3.73 (s, 3H), 3.82 (s, 3H), 4.70 (s, 1H), 6.78-6.88 (m, 3H), 7.29 (m, 1H), 8.55 (s, 1H). ^{13}C NMR (DMSO- d_6 , 75.0 MHz): δ 18.3, 29.8, 54.8, 55.0, 81.8, 112.4, 113.7, 113.9, 117.2, 119.9, 130.1, 144.2, 155.5, 155.6, 159.5, 162.3, 198.1. Anal. calcd. for $\text{C}_{18}\text{H}_{18}\text{N}_2\text{O}_4$ (326.35): C, 66.25; H, 5.56; N, 8.58. Found: C, 66.15; H, 5.40; N, 8.70.

Ethyl N-(5-acetyl-4-(2-chlorophenyl)-3-cyano-6-methyl-4H-Pyran-2-yl)formimidate (compound 5i)

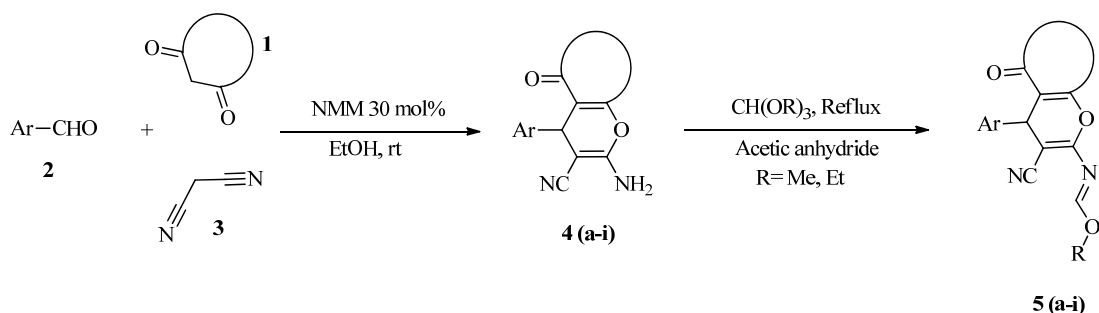
Cream powder, mp: 151-154 °C. IR (KBr) (λ_{\max} , cm^{-1}): 2948, 2212, 1675, 1617. ^1H NMR (DMSO- d_6 , 300 MHz): δ 1.35 (t, 3H), 2.07 (s, 3H), 2.33 (s, 3H), 4.37 (q, $J=7$ Hz, 2H), 5.24

(s, 1H), 7.19-7.28 (m, 3H), 7.38-7.42 (m, 1H), 8.22 (s, 1H). ^{13}C NMR (DMSO- d_6 , 75.0 MHz): δ 13.8, 18.7, 29.3, 37.5, 64.3, 77.0, 77.4, 81.4, 113.3, 116.6, 128.0, 129.2, 130.2, 130.5, 132.9, 139.3, 155.9, 156.4, 159.5, 198.2. Anal. calcd. for $\text{C}_{18}\text{H}_{17}\text{ClN}_2\text{O}_3$ (344.79): C, 62.70; H, 4.97; N, 8.12. Found: C, 62.54; H, 5.01; N, 8.30.

Results and Discussion

The new schiff bases were synthesized in two steps (Scheme 1). At first, 2-amino-4H-Pyran derivatives (4a-i) were synthesized according to a method with a three-component reaction comprising of 1,3-diketone (1), aldehydes (2) and malononitrile (3) in ethanol as solvent using *N*-methylmorpholine as an organocatalyst at room temperature.

The reaction of 3-Nitrobenzaldehyde, malononitrile and dimedone were employed as a template to optimize the reaction conditions (Scheme 2).



Scheme 2. Synthesis of 2-amino-4H-pyran compounds.

Table 1. Screening of catalyst in the formation of 4b.

Entry	(Catalyst mol%)	Time (min) 4b	Yield (%) 4b
1	(NMM 5 mol%)	10	83
2	(NMM 10 mol%)	5	86
3	(NMM 20 mol%)	3	87
4	(NMM 30 mol%)	2	90
5	(NMM 40 mol%)	3	88

Therefore, a mixture of *m*-Nitrobenzaldehyde (1 mmol), malononitrile (1 mmol) and dimedone (1 mmol) in EtOH was stirred for an appropriate time as indicated by TLC using different amounts of NMM. At the end of reaction, the Knoevenagel condensation between aldehydes and malononitrile followed with dimedone resulted in only one product called 4b. To show that NMM is an efficient catalyst, this three components' reaction was accomplished in the absence of catalyst at room temperature for 5 h. This reaction just produced the product of Knoevenagel condensation between aldehydes and malononitrile.

The efficiency of the reaction is mainly affected by the amount of the catalyst (Table 1). The optimal amount of the catalyst was 30 mol% (entry 4); the higher amount of the catalyst did not noticeably increase yield (entry 5).

To generalize this methodology with the optimal conditions, a variety of aldehydes and a series of 1,3-diketone compounds were used to prepare a wide range of 2-amino-4*H*-pyran compounds (Table 2), entries (4a-i) in excellent yields.

After synthesis of 2-amino-4*H*-Pyran, their Schiff base derivatives (5a-i) were prepared by the condensation reaction between the amines (2-amino-4*H*-Pyran derivatives) and the aldehydes (trimethyl or triethyl) orthoformate under reflux conditions and catalytic amount of acetic anhydride. All reactions produced corresponding Schiff-bases (5a-i) in good yield; the results were summarized in Table 3.

All the synthesized and purchased compounds were evaluated for anti-mycobacterial and antifungal activity; the results have been shown in Table 4.

Table 2. Synthesis of 2-amino 4*H*-Pyran derivatives catalyzed by NMM.

Entry	Ar	1, 3-diketone	Product	Time (min)	Yield%	Melting point (°C)	
						Found	Ref
1	<i>p</i> -O ₂ NC ₆ H ₄	Dimedone	4a	10	95	184-187	185-186 (29)
2	<i>m</i> -O ₂ NC ₆ H ₄	Dimedone	4b	2	90	216-218	215-216 (29)
3	<i>p</i> -CH ₃ OC ₆ H ₄	Dimedone	4c	15	88	202-204	200-201 (29)
4	<i>m</i> -CH ₃ OC ₆ H ₄	Dimedone	4d	5	81	189-191	190-191 (29)
5	<i>p</i> -ClC ₆ H ₄	Dimedone	4e	11	88	214-216	215-216 (29)
6	<i>m</i> -ClC ₆ H ₄	Dimedone	4f	8	93	215-217	216-217 (29)
7	C ₆ H ₅	Dimedone	4j	4	92	224-226	225-226 (29)
8	<i>m</i> -CH ₃ OC ₆ H ₄	Acetylacetone	4h	7	88	157-160	
9	<i>o</i> -ClC ₆ H ₄	Acetylacetone	4i	9	88	140-142	

Table 3. Synthesis of Schiff-bases (5a-i) derivatives.

Entry	Amine	Aldehydes	Product	Time (h)	Yield%
1	4a	Trimethylorthoformate	5a	8.5	70
2	4b	Trimethylorthoformate	5b	7	73
3	4c	Trimethylorthoformate	5c	9	66
4	4d	Trimethylorthoformate	5d	7.5	61
5	4e	Triethylorthoformate	5e	8.5	62
6	4f	Trimethylorthoformate	5f	7.5	71
7	4j	Trimethylorthoformate	5j	8	69
8	4h	Trimethylorthoformate	5h	8	65
9	4i	Triethylorthoformate	5i	8.5	64

Table 4. Anti-Mycobacterium and antifungal activity expressed as MIC (μM).

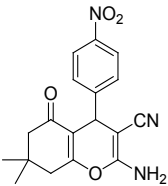
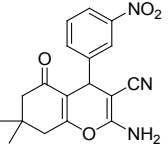
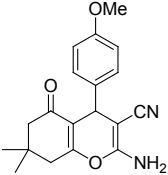
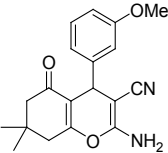
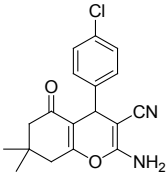
Entry	Code	Structure	BCG MIC ($\mu\text{g/mL}$)		<i>Candida albicans</i> ATCC 10231 MIC ($\mu\text{g/mL}$)	
			24 (h)	48 (h)	24 (h)	48 (h)
1	4a		62.5	62.5	>1000	>1000
2	4b		250	250	>1000	>1000
3	4c		>500	>500	>1000	>1000
4	4d		>500	>500	>1000	>1000
5	4e		250	250	>1000	>1000

Table 4. Continued.

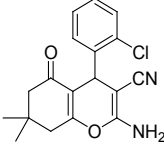
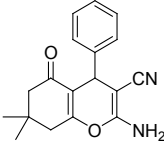
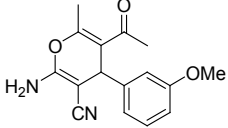
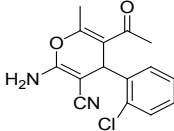
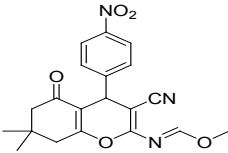
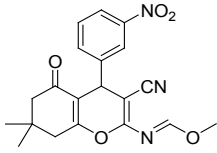
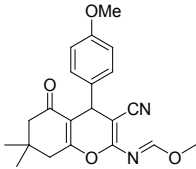
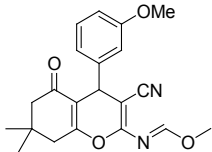
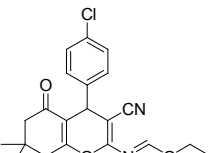
Entry	Code	Structure	BCG MIC ($\mu\text{g/mL}$)		<i>Candida albicans</i> ATCC 10231 MIC ($\mu\text{g/mL}$)	
			24 (h)	48 (h)	24 (h)	48 (h)
6	4f		31.25	31.25	>1000	>1000
7	4g		>500	>500	>1000	>1000
8	4h		250	250	250	500
9	4i		125	125	125	250
10	5a		62.5	62.5	500	>1000
11	5b		62.5	62.5	>1000	>1000
12	5c		>500	>500	>1000	>1000
13	5d		125	125	250	>1000
14	5e		62.5	62.5	>1000	>1000

Table 4. Continued.

Entry	Code	Structure	BCG MIC ($\mu\text{g/mL}$)		<i>Candida albicans</i> ATCC 10231 MIC ($\mu\text{g/mL}$)	
			24 (h)	48 (h)	24 (h)	48 (h)
15	5f		62.5	62.5	>1000	>1000
16	5g		>500	>500	>1000	>1000
17	5h		>500	>500	250	>1000
18	5i		>500	>500	>1000	>1000
ETM			0.78	0.78	-	-
ISO			<0.3	<0.3	-	-
Ketoconazol			-	-	0.75	0.75
AmB			-	-	0.75	0.75
DMSO			5%v/v	5%v/v	5%v/v	10%v/v

MIC: Minimum Inhibitory Concentration; ETM: Ethambutol; ISO: Isoniazid; AMB: amphotericin B.

As indicated in Table 4, the majority of the MIC results for the tested compounds showed a potentially good effect against *Mycobacterium bovis* (*M. bovis*). The compound 4f showed the highest antimycobacterial activity (31.25 $\mu\text{g/mL}$) and compounds 4a, 5a, 5b, 5e, 5f demonstrated the high activity against *Mycobacterium* (62.5 $\mu\text{g/mL}$) (Table 4). It seems the activity of compounds depends on electron donor and also the position of substituents in aromatic ring.

It is evident that the rings substituted with electron withdrawing groups such as NO_2 and Cl showed better activity than donor substituent such as OMe in 4c or unsubstituted phenyl ring compound such as 4j and 5j compounds.

Presence of Cl substitution in 2-position in

benzene ring (compound 4f) resulted in better activity against *Mycobacterium* than compound 4e which include Cl substitution in 4-position.

In schiff bases fused 4H-Pyran compounds, most of 4H-Pyran s which synthesized from dimedone such as 4a-4j, showed better antimycobacterial activity compared to those which were synthesized from acetylacetone such as 4h and 4i. Moreover, to assess antimicrobial activity of these compounds, antifungal activity was evaluated. *Candida albicans* (*C.albicans*) is introduced as a good candidate for antifungal activity tests. Mainly, the treated compounds showed poor activity against *C.albicans*, compounds 4h and 4i, which were synthesized from acetylacetone, scored the highest antifungal

activity.

Conclusion

In conclusion, a series of biologically and pharmacologically active 4*H*-Pyran derivatives (4a-i) have been synthesized under simple conditions and in the attendance of NMM as a catalyst, subsequently new high-yield Schiff-bases (5a-i) containing 4*H*-Pyran core synthesized. The anti-mycobacterial and antifungal test results demonstrated that the majority of the synthesized compounds were active against *M. bovis* and they have poor activity against *C. albicans*.

Acknowledgment

Financial support of this work by the Kharazmi University of Tehran (Faculty of Chemistry) and Pasteur Institute of Iran (Drug Design and Bioinformatics Unit), is gratefully acknowledged.

References

- (1) Xu ZQ, Pupek K, Suling WJ, Enache L and Flavin MT. Pyran coumarin, a novel anti-TB pharmacophore: Synthesis and biological evaluation against *Mycobacterium tuberculosis*. *Bioorg. Med. Chem.* (2006) 14: 4610-26.
- (2) Bacelar AH, Carvalho MA and Proença MF. Synthesis and *in-vitro* evaluation of substituted pyrimido [5, 4-d] pyrimidines as a novel class of Anti-mycobacterium tuberculosis agents. *Eur. J. Med. Chem.* (2010) 45: 3234-9.
- (3) Ballell L, Field RA, Duncan K and Young RJ. New small-molecule synthetic antimycobacterials. *Antimicrob. Agents Chemother.* (2005) 49: 2153-63.
- (4) Nayyar A and Jain R. Recent advances in new structural classes of anti-tuberculosis agents. *Curr. Med. Chem.* (2005) 12: 1873-86.
- (5) Weber L. The application of multi-component reactions in drug discovery. *Curr. Med. Chem.* (2002) 9: 2085-93.
- (6) Schreiber SL. Target-oriented and diversity-oriented organic synthesis in drug discovery. *Science* (2000) 287: 1964-9.
- (7) Andreani L and Lapi E. Aspects and orientations of modern pharmacognosy. *Boll. Chim. Farm.* (1960) 99: 583-6.
- (8) Khafagy MM, Abd El-Wahab AH, Eid FA and El-Agrody AM. Synthesis of halogen derivatives of benzo[h]chromene and benzo[a]anthracene with promising antimicrobial activities. *Farmaco* (2002) 57: 715-22.
- (9) Kumar D, Reddy VB, Sharad S, Dube U and Kapur S. A facile one-pot green synthesis and antibacterial activity of 2-amino-4*H*-Pyrans and 2-amino-5-oxo-5,6,7,8-tetrahydro-4*H*-chromenes. *Eur. J. Med. Chem.* (2009) 44: 3805-9.
- (10) Mohr SJ, Chirigos MA, Fuhrman FS and Pryor JW. Pyran copolymer as an effective adjuvant to chemotherapy against a murine leukemia and solid tumor. *Cancer Res.* (1975) 35: 3750-4.
- (11) Smith PW, Sollis SL, Howes PD, Cherry PC, Starkey ID, Cobley KN, Weston H, Scicinski J, Merritt A and Whittington A. Dihydropyran carboxamides related to zanamivir: A new series of inhibitors of influenza virus sialidases. 1. Discovery, synthesis, biological activity, and structure-activity relationships of 4-guanidino- and 4-amino-4*H*-Pyran -6-carboxamides. *J. Med. Chem.* (1998) 41: 787-97.
- (12) Martínez-Grau A and Marco J. Friedländer reaction on 2-amino-3-cyano-4*H*-Pyrans: Synthesis of derivatives of 4*H*-Pyran [2, 3-b] quinoline, new tacrine analogues. *Bioorg. Med. Chem. Lett.* (1997) 7: 3165-70.
- (13) Bianchi G and Tava A. Synthesis of (2*R*)-(+)-2,3-Dihydro-2,6-dimethyl-4*H*-Pyran-4-one, a homologue of pheromones of a species in the Hepialidae family. *Agric. Biol. Chem.* (1987) 51: 2001-2.
- (14) Dell C and Smith C. Antiproliferative derivatives of 4*H*-naphtho [1, 2-b] Pyran and process for their preparation. *EP537949* (1993) 21.
- (15) Konkoy CS, Fick DB, Cai SX, Lan NC and Keana JFW. Substituted 5-oxo-5,6,7,8-tetrahydro-4*H*-1-benzopyrans and benzothiopyrans and their use as potentiators of AMPA. PCT Int. Appl. WO Patent 0075123, 2000. *Chem. Abstr.* (2001) 134: 29313.
- (16) Singh K, Singh J and Singh H. A synthetic entry into fused Pyran derivatives through carbon transfer reactions of 1, 3-oxazinanes and oxazolines with carbon nucleophiles. *Tetrahedron* (1996) 52: 14273-80.
- (17) Hatakeyama S, Ochi N, Numata H and Takano S. A new route to substituted 3-methoxycarbonyldihydropyrans; enantioselective synthesis of (-)-methyl elenolate. *J. Chem. Soc. Chem. Comm.* (1988) 17: 1202-4.
- (18) Armesto D, Horspool WM, Martin N, Ramos A and Seoane C. Synthesis of cyclobutenes by the novel photochemical ring contraction of 4-substituted 2-amino-3,5-dicyano-6-phenyl-4*H*-Pyran s. *J. Org. Chem.* (1989) 54: 3069-72.
- (19) Mori K, Audran G and Monti H. The first synthesis of coniochaetones A and B: Two benzopyranone derivatives. *Synlett* (1998): 259-60.
- (20) Saleshier FM, Suresh S, Anitha N, Karim J and Divakar M. Design, docking and synthesis of some 6-benzimidazolyl Pyran s and screening of their anti tubercular activity. *Eur. J. Exp. Biol.* (2011) 1: 150-9.
- (21) Gudipati R, Anreddy RNR and Manda S. Synthesis, characterization and anticancer activity of certain 3-{4-(5-mercapto-1,3,4-oxadiazole-2-yl) phenylimino} indolin-2-one derivatives. *Saudi Pharm. J.* (2011) 19: 153-8.
- (22) Konkoy C, Fick D, Cai S, Lan N and Keana J. In PCT

- Int. Appl. WO 0075123, 2000; *Chem. Abstr.* (2001) 134: 29313a.
- (23) Vazzana I, Terranova E, Mattioli F and Sparatore F. Aromatic schiff bases and 2,3-disubstituted-1,3-thiazolidin-4-one derivatives as antiinflammatory agents. *ARKIVOC* (2004) 364: 374.
- (24) Pandeya S, Sriram D, Nath G and DeClercq E. Synthesis, antibacterial, antifungal and anti-HIV activities of schiff and Mannich bases derived from isatin derivatives and N-[4-(4'-chlorophenyl) thiazol-2-yl] thiosemicarbazide. *Eur. J. Pharm. Sci.* (1999) 9: 25-31.
- (25) Armesto D, Horspool WM, Martin N, Ramos A and Seoane C. Synthesis of cyclobutenes by the novel photochemical ring contraction of 4-substituted 2-amino-3, 5-dicyano-6-phenyl-4H-Pyran s. *J. Org. Chem.* (1989) 54: 3069-72.
- (26) Patel IJ and Parmar SJ. Synthesis and studies of novel optically active Schiff's base derivatives and their antimicrobial activities. *J. Chem.* (2010) 7: 617-23.
- (27) Del Rayo Camacho-Corona M, Ramírez-Cabrera MA, Santiago OG, Garza-González E, Paz Palacios I and Luna-Herrera J. Activity against drug resistant-tuberculosis strains of plants used in Mexican traditional medicine to treat tuberculosis and other respiratory diseases. *Phytother. Res.* (2008) 22: 82-5.
- (28) Cuenca-Estrella M, Lee-Yang W, Ciblak MA, Arthington-Skaggs BA, Mellado E, Warnock DW and Rodriguez-Tudela JL. Comparative evaluation of NCCLS M27-A and EUCAST broth microdilution procedures for antifungal susceptibility testing of *Candida* species. *Antimicrob. Agents* (2002) 46: 3644-7.
- (29) Guo RY, An ZM, Mo LP, Wang RZ, Liu HX, Wang SX and Zhang ZH. Meglumine: A novel and efficient catalyst for one-pot, three-component combinatorial synthesis of functionalized 2-amino-4 H-Pyran s. *ACS Comb. Sci.* (2013) 15: 557-63.

This article is available online at <http://www.ijpr.ir>
