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SYNTHESIS OF THIAZOLO [3, 2-A] BENZIMIDAZOLE DERIVATIVES USING MICROWAVE AS GREEN SYNTHETIC TECHNIQUE AND THEIR ANTIMICROBIAL ACTIVITIES

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Abstract:
In the present study, 2-mercapto benzimidazole (**1**) was condensed with substituted acetophenone (**2**) to give 2-Benzimidazolylthioacetophenone derivatives 3(a-l). This on microwave radiation in acetic anhydride, cyclized to corresponding Thiazolo [3,2-a]benzimidazole derivatives 4(a-l). Synthesized compounds were characterized by spectroscopic methods such as IR, 1HNMR, and Mass spectrometry. All the synthesized compounds were evaluated for in-vitro activities against a panel of Gram-positive and Gram-negative bacteria. The entire compound exhibited excellent to moderate activity against all pathogens.
Keywords: Microwave, Thiazolo [3, 2-a] benzimidazole, spectroscopy, antibacterial, antifungal.

Introduction

Heterocyclic compounds (HC) are key structures of many natural products. Multiple structures of HC are the core elements extensively in numerous natural and pharmaceutical products with significant biological activities. Almost every drug contains HC as the main structure. In many natural molecules including DNA, RNA, and vitamins, chlorophyll heterocyclic ring is the main contributor [i-iii]. HC contains at least one nitrogen, oxygen, and sulfur as a hetero atom in their cyclic structure. Other hetero-atoms such as phosphorus, magnesium, iron, selenium, etc. are also considered as common HC [iv-v]. These hetero atoms act as functional groups for the whole entity. The availability of lone pairs of electrons and the electromagnetic difference between hetero atoms to carbon considerably impact the nature, reactivity, and structural modifications of heterocycles. HC has better chemical adaptability and responds greatly to the diverse demands of biological systems. During drug design, unique structural and electronic properties of HC allow them to interact with various receptors and enzymes in the body [vi-viii]. The difference in polarity of hetero atom favors van der Waals forces, hydrophobic effects, hydrogen bonding formation, π stacking interactions, and dipole-dipole interactions allowing the compound to improve ADMET profile, physicochemical and pharmacological properties. In the last few decades, fused heterocyclic

derivatives have become an important scaffold due to promising biological and medicinal activity [ix].

Benzimidazole, a hybrid fused heterocyclic bi-cyclical compound composed of benzene and imidazole is an important contributor to the field of industrial, biological, and medicinal chemistry, drug discovery, and clinical applications including pharmacological activities. Histidine, purines and a part of vitamin B_{12} are some of natural sources of benzimidazole where as fenbendazole, oxfenbendazole, thiabendazole, mebendazole, omeprazole, lansoprazole and pantoprazole are drugs containing benzimidazole as core structure. The minimal toxicity properties of benzimidazole & its derivatives have made them promising theoretic agents for antimicrobial, anti-tuberculosis, anti-viral, anti-ulcer, anti-inflammatory, anti-diabetic, anti-consultant, anti-hypertensive and anti-malarial activity [x-xiii]. Dyestuff, sanitizers, corrosion inhibitors, antioxidants, and copolymer synthesis are additional well-known applications [xiv].

The synthetic methodology for organic synthesis is continuously developing in accordance with engineering. This has a great impact not only on the economic field but also on the environmental aspect, which is very important for future sustainability considerations. The term Green Chemistry uses various potent techniques for making chemical substances in a sustainable way by minimizing the hazardous footprint. Techniques include Microwave, Ultrasound, Ball & Milling, Photocatalysis, Continuous Flow Reactor, Magnetic field-assisted synthesis, Hydro (solvo) thermal synthesis [xv].

In the conventional synthesis method one usually needs longer heating time at higher temperatures which also generates unwanted impurities, tedious apparatus setup results in higher process costs, use of excessive solvents/ reagents leads to environmental pollution. The growth of green chemistry approaches holds significant potential for a reduction of the by-products, waste production, high yield, and lowering of energy costs. Microwave-assisted synthesis provides clean synthesis with the advantage of enhanced reaction rates, higher yields, greater selectivity, and economics for the synthesis of a large number of organic molecules [xvi-xvii].

Experimental

Materials and methods

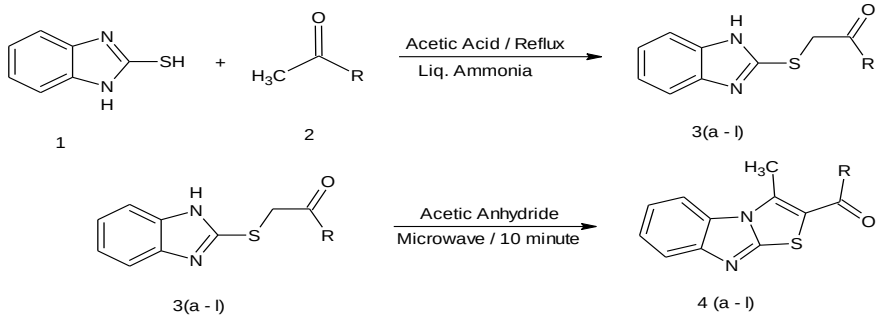
All reagents and solvents were used of commercial grade without any further purification. The progress of reactions was checked by thin layer chromatography on an alumina plate coated with silica gel60 F_{254}, 0.25 mm thickness (Merck), and the developed chromatograms were visualized under an Ultraviolet light cabinet (254 nm). Melting points were determined in an open capillary using the Metteler Toledo MP50 melting point apparatus. IR spectra were recorded on the Make Bruker FTIR spectrometer using potassium bromide pellets in the range of 4000 to 400 cm^{-1}. Mass spectra were recorded on Agilent Technologies Mass spectrometer. ^1H NMR spectra were recorded on Bruker Avance 400 MHz spectrometer (Bruker Scientific Ltd Switzerland) using DMSO-d_6 as solvent and Tetramethyl silane as internal standard. HPLC purity was performed using Waters HPLC.

General Procedures for Synthesis of 2-Benzimidazolylthioacetophenone Derivatives (3a-j): To the mixture of 2-mercaptobenzimidazole (1, 10 mmol) and substituted acetophenone (2, 15 mmol) in acetic acid (10 ml) added few drops of concentrated sulfuric acid. The reaction was refluxed for 150 to 180 minutes. The progress of the reaction was confirmed by TLC. After completion of the reaction, the reaction mixture was cooled and poured into ice-cold water. Neutralize the reaction mass using liquor ammonia. Filter the crude solid and suck it dry. Crystallize the crude intermediate in ethanol to get a pure compound. Yield 80 – 85%.

General Procedures for Synthesis of 2-Aroyl-3-methylthiazolo [3, 2-a] benzimidazole (4a-l): Mixture of 3(a-l) (5 mmol) and acetic anhydride (15 ml) was placed in Pyrex

glassware and irradiate in microwave oven for 10 minutes. The reaction mass was quenched in cold water, neutralized by ammonium hydroxide and the compound was collected by filtration. The crude solid was purified in ethanol. Yield 65 to 75%.

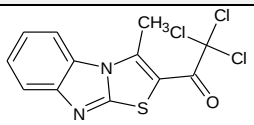
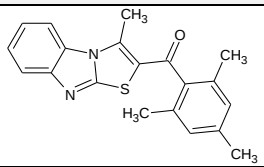
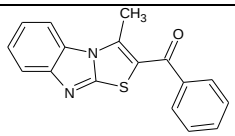
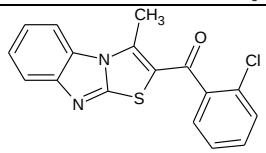
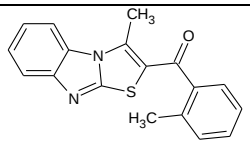
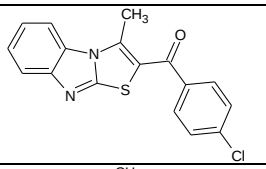
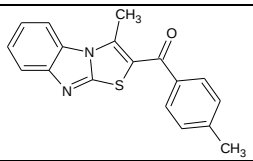
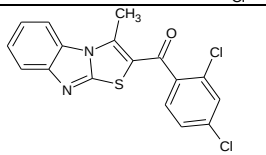
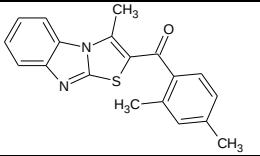
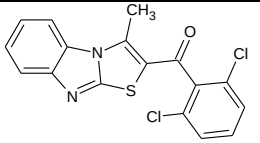
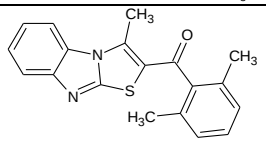
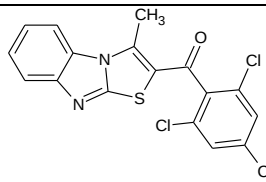
Reaction Scheme:



Scheme-1:

4a)2,2,2-trichloro-1-(3-methyl[1,3]thiazolo[3,2-a]benzimidazol-2-yl)ethenone: Pale yellow solid, M.P.: 147-149°C, IR (KBr, cm-1): 3097(Ar-H str.), 2922 (C-H str.), 1697 (C=O str.), 1592 (Ar-C=C str.), 1449 (C-H bend), 1H NMR: δ 3.79-3.83 (s, 3H, Methyl), 7.08-7.13 (dd, 2H, Ar-H), 7.53-7.58 (dd, 1H, Ar-H),7.73 (dd, 1H, Ar-H), ESI-MS (m/z) = 333.85(M^+) Actual =333.62, HPLC purity=97.58 % area.

Table 01: Yield details

Entry	Product	Yield% (65-75)	Entry	Product	Yield % (65-75)
4a		**73**	**4g**		**65**
4b		**72**	**4h**		**71**
4c		**70**	**4i**		**66**
4d		**72**	**4j**		**72**
4e		**68**	**4k**		**69**
4f		**75**	**4l**		**73**

4b)(3-methyl[1,3]thiazolo[3,2-a]benzimidazol-2-yl)(phenyl)methanone: Offwhite solid, M.P.=124-128 °C, IR (KBr, cm-1): 3098(Ar-H str.), 2921 (C-H str.), 1698 (C=O str.), 1610 (Ar-C=C str.), 1450(C-H bend), 1H NMR: δ 2.61 (s, 3H, Methyl), 7.26-7.58 (dd, 2H, Ar-H), 7.60 (dd, 1H, Ar-H), 7.88-7.91 (dd, 1H, Ar-H), 7.99-8.03 (dd, 1H, Ar-H), 8.13-8.14 (dd, 2H, Ar-H), 9.22-9.55 (dd, 1H, Ar-H), ESI-MS (m/z) = 294.08 (M^+) Actual =292.35, HPLC purity=98.31% area.

4c) (2-methylphenyl)(3-methyl[1,3]thiazolo[3,2-a]benzimidazol-2-yl)methanone: Light Brown solid, M.P.= 146-148 °C, IR (KBr, cm-1): 3091(Ar-H str.), 2941 (C-H str.), 1706 (C=O str.), 1597 (Ar-C=C str.), 1429 (C-H bend), 1H NMR: δ 2.11 (s, 3H, p-Methyl), 2.57 (s, 3H, Methyl), 7.11-7.13 (dd, 2H, Ar-H), 7.14-7.16 (dd, 2H, Ar-H), 7.68-7.73 (dd, 2H, Ar-H), 7.84-7.91 (dd, 2H, Ar-H), ESI-MS (m/z) = 307.83 (M^+) Actual =306.38, HPLC purity=98.84% area.

4d) (4-methylphenyl)(3-methyl[1,3]thiazolo[3,2-a]benzimidazol-2-yl)methanone: Brown solid, M.P.= 102-104 °C, IR (KBr, cm-1): 2981(Ar-H str.), 2937 (C-H str.), 1723 (C=O str.), 1606 (Ar-C=C str.), 1456 (C-H bend), 1H NMR: δ 2.71-2.77 (s, 3H, p-Methyl), 2.86-4.17 (s, 3H, Methyl), 6.01-6.02 (ddd, 2H, Ar-H), 6.89-6.91(dd, 2H, Ar-H), 7.03-7.05 (dd, 2H, Ar-H), 7.72-7.78 (ddd, 4H, Ar-H), ESI-MS (m/z) = 308.90 (M^+) Actual =306.38, HPLC purity=98.36% area.

4e)(2,4-dimethylphenyl)(3-methyl[1,3]thiazolo[3,2-a]benzimidazol-2-yl)methanone: White solid, M.P.=110-113 °C, IR (KBr, cm-1): 3019 (Ar-H str.), 2959 (C-H str.), 1717 (C=O str.), 1612 (Ar-C=C str.), 1422 (C-H bend), 1H NMR: δ 2.24 (s, 3H, o-Methyl), 2.31 (s, 3H, p-Methyl), 2.52 (s, 3H, Methyl), 7.04-7.16 (dd, 4H, Ar-H), 7.43-7.49 (dd, 1H, Ar-H), 7.86-7.95 (dd, 2H, Ar-H), ESI-MS (m/z) = 320.45 (M^+) Actual =320.40, HPLC purity=97.71% area.

4f)(2,6-dimethylphenyl)(3-methyl[1,3]thiazolo[3,2-a]benzimidazol-2-yl)methanone:White solid, M. .P.=91-93°C, IR (KBr, cm-1): 3081(Ar-H str.), 2952 (C-H str.), 1714 (C=O str.), 1598 (Ar-C=C str.), 1428 (C-H bend), 1H NMR: δ 2.37 (s, 6H, Methyl), 2.62 (s, 3H, Methyl), 7.16-7.36 (ddd, 5H, Ar-H), 7.79-7.92 (dd, 2H, Ar-H), ESI-MS (m/z) = 320.40 (M^+) Actual =321.48, HPLC purity=97.92% area.

4g)(3-methyl[1,3]thiazolo[3,2-a]benzimidazol-2-yl)(2,4,6-trimethylphenyl)methanone:Off white solid, M.P.=102-104°C, IR (KBr, cm-1): 3097(Ar-H str.), 2922 (C-H str.), 1697(C=O str.), 1610 (Ar-C=C str.), 1449 (C-H bend), 1H NMR: δ 2.31 (s, 3H, p-Methyl), 2.56 (s, 6H, o- Methyl), 2.81 (s, 3H, Methyl), 7.09-7.83 (ddd, 6H, Ar-H), ESI-MS (m/z) = 333.85 (M^+) Actual =333.43, HPLC purity=97.20% area.

4h(2-Chlorophenyl)(3-methyl[1,3]thiazolo[3,2-a]benzimidazol-2-yl methanone: Yellowish residual solid, M.P.=197-199°C, IR (KBr, cm-1): 3097(Ar-H str.), 2926 (C-H str.), 1697 (C=O str.), 1610 (Ar-C=C str.), 1449 (C-H bend), 1H NMR: δ 2.61 (s, 3H, Methyl), 7.11-7.26 (dd, 2H, Ar-H), 7.42-7.60 (ddd, 3H, Ar-H), 7.64-7.83 (ddd, 3H, Ar-H), ESI-MS (m/z) = 327.16 (M^+) Actual =326.80, HPLC purity=98.09% area.

4i) (4-Chlorophenyl) (3-methyl[1,3]thiazolo[3,2-a]benzimidazol-2-yl)methanone: Yello residual solid, M.P.=201-204 °C, IR (KBr, cm-1): 3091(Ar-H str.), 2931 (C-H str.), 1706 (C=O str.), 1596 (Ar-C=C str.), 1426 (C-H bend), 1H NMR: δ 2.69 (s, 3H, Methyl), 7.16-7.30 (dd, 2H, Ar-H), 7.49-7.63 (dd, 2H, Ar-H), 7.69-7.93 (ddd, 4H, Ar-H), ESI-MS (m/z) = 328.23 (M^+) Actual =326.80, HPLC purity=98.09% area.

4j)(2,4-Dichlorophenyl)(3-methyl[1,3]thiazolo[3,2-a]benzimidazol-2-yl)methanone: Pale yellow solid, M.P.=211-213 °C, IR (KBr, cm-1): 3096(Ar-H str.), 2928 (C-H str.), 1701 (C=O str.), 1602 (Ar-C=C str.), 1428 (C-H bend), 1H NMR: δ 2.83 (s, 3H, Methyl), 7.03-7.48 (ddd, 5H, Ar-H), 7.72-7.86 (dd, 2H, Ar-H), ESI-MS (m/z) = 363.16 (M^+) Actual =361.24, HPLC purity=98.16% area

4k)(2,6-Dichlorophenyl)(3-methyl[1,3]thiazolo[3,2-a]benzimidazol-2-yl)methanone: Yellow semi solid, M.P.=184-186°C, IR (KBr, cm-1): 3066(Ar-H str.), 2962 (C-H str.), 1687 (C=O str.), 1562 (Ar-C=C str.), 1410 (C-H bend), 1H NMR: δ2.73 (s, 3H, Methyl), 6.93-7.48 (ddd, 5H, Ar-H), 7.66-7.79 (dd, 2H, Ar-H), ESI-MS (m/z) = 362.83 (M^+) Actual =361.24, HPLC purity=98.69% area.

4l)(2,4,6-Triichlorophenyl)(3-methyl[1,3]thiazolo[3,2-a]benzimidazol-2-yl)methanone: Yellow residue, M.P.=136-138°C, IR (KBr, cm-1): 3063 (Ar-H str.), 2964 (C-H str.), 1681 (C=O str.), 1548 (Ar-C=C str.), 1429 (C-H bend), 1H NMR: δ2.88 (s, 3H, Methyl), 7.08-7.22 (dd, 2H, Ar-H), 7.58-7.72 (dd, 2H, Ar-H), 7.76-7.92 (dd, 2H, Ar-H), ESI-MS (m/z) = 396.18 (M^+) Actual =395.69, HPLC purity=98.53% area.

Biological Evolution

The antimicrobial activity of synthesized compounds was carried out by broth dilution method and the National Committee for Clinical Laboratory Standards (NCCLS) was followed. Antibacterial activity was screened against two Gram-negative *E. Coli* MTCC-443 and *P. Aeruginosa* MTCC 1688 and two Gram positive*S. Aurous* MTCC 96 and*S. Progenies* MTCC 442 bacteria using Chloramphenicol and Ciprofloxacin as the standard antibacterial drugs.

Antifungal activity was screened against three fungal species *C. Albicans* MTCC 227, *A. Niger* MTCC 282, and *A.Clavatus* MTCC 1323 where Nystatin and Griseofulvin were used as the standard antifungal drugs.

The strains employed for the activity were procured from the Institute of Microbial Technology, Chandigarh. Mueller Hinton broth was used as the nutrient medium to grow and dilute the drug suspension for the test. DMSO was used as the diluent to get the desired concentration of compounds to test upon the standard bacterial strains.

In vitro antibacterial activity

For E.Coli, compounds 4f and 4i each have activity MIC-62.50 µg/ml moderate when compared with standard chloramphenicol (MIC-50 µg/ml). For P.Aeruginosa, the whole series shows moderate results when compared with chloramphenicol (MIC-50 µg/ml) and ciprofloxacin (MIC-25 µg/ml). For S. Aurous, compounds 4b and 4e show equal activity MIC-62.50 µg/ml compared with standard chloramphenicol (MIC-50 µg/ml) and ciprofloxacin (MIC-25 µg/ml). For S.Pyogenus, compound 4a to 4l had moderate activity as shown in Table 02.

Table 02: Antibacterial and Antifungal Activity

Antibacterial Activity (Mic)					**Antifungal Activity (Mic)**		
Minimal Inhibition Concentration (µgm/ml)							
Entry	*E.Coli*	*P. Aeruginosa*	*S. Aurous*	*S. Progenies*	*C. Albicans*	*A. Niger*	*A. Clavatus*
4a	250	200	100	200	500	1000	1000
4b	200	250	**62.5**	125	500	500	>1000
4c	100	200	125	100	1000	1000	1000
4d	200	125	200	125	500	500	1000
4e	250	250	**62.5**	100	1000	500	500
4f	**62.5**	125	100	200	1000	1000	1000
4g	100	100	200	250	**250**	**500**	**500**
4h	125	200	125	100	550	500	1000
4i	**62.5**	100	200	100	1000	1000	1000

4j	125	100	125	200	500	1000	1000
4k	200	100	200	225	1000	500	500
4l	100	125	100	200	500	1000	500
Chloramphenicol	50	50	50	50	NA	NA	NA
Ciprofloxacin	25	25	50	50	NA	NA	NA
Nystatin	NA	NA	NA	NA	100	100	100
Griseofulvin	NA	NA	NA	NA	500	100	100

In vitro antifungal activity

Whole series 4(a-l) shows good antifungal activity when compared against standard Griseofulvin (MIC-250 µg/ml) and Nystatin (MIC-100 µg/ml). However, compound 4g had good antifungal activity and could be a potent antifungal drug.

Result and discussion

Chemistry

2-Mercaptobenzimidazole (1) and substituted acetophenone (2) condense in acidic conditions at reflux to give 2-Benzimidazolylthioacetophenone derivatives (3). Microwave radiation of compound 3 in acetic anhydride gives a corresponding series of 2-Aroyl-3-methylthiazolo [3, 2-a] benzimidazole (4a-l) in good yield. Microwave as Green Technique emphasizes less time, high yield, simple reaction setup, ideal reaction temperature with homogeneity of the heating, and less effluent generation. Reaction progress and purity of compounds were checked using thin-layer chromatography, where spots were visualized under ultraviolet light (254 nm). Synthesized series 4(a-l) was accomplished by IR, NMR, and Mass spectra analysis. Synthesized compounds were screened for anti-microbial. Compounds 4(b, e, f, and i) showed good antibacterial activity. Compound 4g had potent antifungal activity.

Conclusion

A series 2-Aroyl-3-Methylthiazolo [3, 2-A] benzimidazole 4(a-l) was synthesized using a microwave as a green synthetic technique. The microwave way assists with mild reaction conditions, less reaction time affording good to moderate yield, less effluent generation, and no tedious apparatus. Obtained compounds were acknowledged by IR, 1H NMR, and Mass spectroscopy. The obtained compounds were screened for antibacterial and antifungal activity. Compound **4(b, e, f, and i)** had good antibacterial activity when compared against standard chloramphenicol and ciprofloxacin. Compounds 4(a-l) had good antifungal screening but molecule 4g could be a potent antifungal drug. All the synthesized compounds show excellent to moderate activity against all pathogens and are promising lead molecules for further optimization using modeling techniques.

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Conflict of interest

The authors declare that no conflict of interest.

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