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Construction, characterization and antibacterial activity of pyrazolone, thiohydantoin and their derivatives

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Heterocyclic organic compounds play pivotal roles in drug synthesis and continue to remain a fundamental area of research interest. The present study reports the synthesis, characterization and antibacterial activities of synthesized organic compounds containing pyrazolone and thiohydantoin moieties. Pyrazolone and thiohydantoin scaffolds have been constructed using well-defined methods and their derivatives have been synthesized Structural elucidation has been achieved via IR and NMR spectroscopy techniques. The synthesized compounds have been screened for antimicrobial activity at 50 µg/mL concentration, against three pathogenic micro-organisms, viz: *E. coli, K. pneumonia and P. aeruginosa,* using the agar diffusion cup plate method. Ciprofloxacin is used as control. Interestingly, all the compounds have exhibited antimicrobial activities. Compound 2b have shown the highest sensitivity against *P. aeruginosa* with zone of inhibition (ZOI) of 13 mm. Compounds 1a and 2a have shown highest activity against *K.pneumonia* with ZOI 11 mm each. Compound 2a has shown highest sensitivity against *E. coli* with ZOI of 8 mm. These findings indicate that the synthesized compounds are pure and possess therapeutic properties.

Keywords: Antibacterial activity, Hydantoin, Pyrazole, Pyrazolone, Thiohydantoin

The discovery of the pharmacological potency of heterocyclic compounds has completely revolutionized medicinal chemistry and interest of medicinal and organic chemists is focused on syntheses of heterocyclic compounds which may be used as precursors/ lead compounds during drug production. Over the years, heterocyclic compounds have proven to be a perfect alternative source for drug synthesis, and a bewildering number of drugs have been synthesized using heterocyclic compounds as precursors.[1] Derivatives of pyrazole and thiohydantoin, among other heterocyclic compounds, have also exhibited numerous biological activities[1], and are thus used in the synthesis of drugs. The pyrazole ring (Fig. 1a) consists of a doubly unsaturated five-member with two adjacent nitrogen atoms of which one is basic (pyrimidine type –N=) and the other is neutral in nature. Pyrazolones (Fig. 1b) are the oxo derivatives of doubly unsaturated 5-membered heterocyclic compounds having two adjacent nitrogen atoms[2]. Pyrazole and its analogues have found use as building blocks in organic synthesis for designing drugs and agrochemicals; and as bi-functional ligands for metal catalysis. This class of heterocyclic compound have been known to exhibit antimicrobial, analgesic, anti-inflammatory[3], antipyretic[4], antimycobacterial[5], anticancer[6], gastric secretion stimulatory[7], anticonvulsant[7] , antimalarial[7], anti-HIV[8], anti-diabetic[9], anti-hyperlipidemic[7], and immunosuppressive activities[10]. 5,5-Diphenyl-imidazolidine-2,4-dione (Fig 1c) (also known as phenytoin or hydantoin) is a 5-membered ring with two nitrogen atoms at position 1 and 3 in the ring. The ring contains two carbonyls one between the nitrogen atoms, another at position-4. The five positions of the ring are numbered and, as such, there are four points of functionality viz: at positions 1, 3 and two at the position 5.

The hydantoin ring is planar and its inclusion in a compound imparts a good deal of rigidity to the structure. This rigidity is imparted by the two amides in the ring which give rise to a resonance form with double bond character at positions 1-2 and 3-4. It is not hard to imagine some double bond character also at the 2-3 position, whereupon the hydantoin ring can be seen to have highly planar and rigid properties that

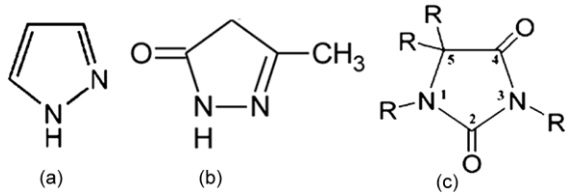


Fig. 1 — (a) Pyrazole ring, (b) pyrazolone and (c) hydantoin

make for a tough scaffold around which the rest of a compound may be constructed. For this reason, they have become popular start materials to synthesize compound libraries for drug design and discovery. Phenytoin is used to manage arhythmia and migraine headaches and facial nerve pain[11]. Thiohydantoins are sulfur analogs of hydantoins. 2-thiohydantoins are most notably known compared to other thiohydantions due to their numerous biological applications[12,13], anticarcinogenic[14], antimutagenic[15,16], antithyroidal[17], antiviral (e.g., against herpes simplex virus, HSV)[18], human immunodeficiency virus (HIV)[19] and tuberculosis[20], antimicrobial (antifungal and antibacterial), anti-ulcer and anti-inflammatory agents, as well as pesticides[14]. The exponential rate of increase in demand for pharmacologically active heterocyclic analogues has prompted our decision to synthesize, characterize and investigate the pharmacological potency of compounds containing pyrazolone and thiohydantoin moieties, since these compounds are known for their biological activities.

Experimental Details

Reagents used were analytical grade, with no additional purification. Melting point determination was achieved by open capillary method. Purity of the compounds was checked with the help of thin layer chromatography. The chromatograms were viewed in a UV chamber. Structural determination was achieved by ^{1}H-NMR, and IR spectroscopy techniques.

Synthesis of 5-methyl-2,4-dihydro-3H-pyrazol-3-one (1)

Ethyl acetoacetate (0.05 mol) was measured and transferred into a clean 250 mL conical flask. Hydrazine hydrate (0.1 mol) in 40 mL ethanol was added drop by drop to the content of the conical flask while stirring. The temperature of the flask content increased during addition and it was regulated at 60°C. After complete addition, the mixture was uniformly stirred for 1 h so that crystalline deposits separated out, then after it was placed in an ice-bath for 1 h for complete crystallization. After crystallization, the mixture was filtered, washed with ice cold alcohol, air-dried and stored for further synthesis.

Yield: 74%, White colored compound, m.p: 216-218°C IR (KBr, cm^{-1}): 3440 (N-H str.), 2949 (C-H str., CH_3), 1709(C=O str.), 1616 (C=N str.), ^{1}H NMR (DMSO) δ5.206(s 1H, NH), δ 2.51 (s, 3H, CH_3).

Synthesis of 5-methyl-2-[(4-methylphenyl) sulfonyl]-2,4-dihydro-3H-pyrazol-3-one (1a)

Synthesized compound 1 (0.01 mol) was weighed and dissolved in 20 mL ethanol in a clean 250 mL single neck round bottom flask fitted with a reflux condenser. To this, pyridine (1 mL) and *p*-toluene sulfonyl chloride (0.01 mol) were added respectively, after which the mixture was refluxed for 18 h. The mixture was left to cool down to room temperature then slowly poured into ice. The resulting precipitate was filtered through a Buchner funnel with suction, and re-crystallized from ethanol.

Yield: 62%, Pale pink colored compound, m.p: 116-117.4°C IR (KBr, cm^{-1}): 2949 (C-H str., CH_3), 1709(C=O str.), 1616 (C=N str.), ^{1}H NMR (DMSO) δ 2.150 (s, 3H, CH_3), δ 2.420 (s, 3H, CH_3), δ 7.456-7.477 (d, 2H, Ar), δ 7.752-7.772 (d, 2H, Ar), δ 5.707 (s, 2H, CH_2)

Synthesis of 5,5-diphenyl-2-thioxoimidazolidin-4-one (2)

Benzil (0.05 mol), thio-urea (0.05 mol), 20 mL of 30% sodium hydroxide solution and 75 mL ethanol were taken in a clean 250 mL single neck round bottom flask fitted to a reflux condenser. The mixture was subjected to 4 h reflux then it was left to cool down to room temperature. It was poured into 125 mL of cold water, mixed thoroughly, allowed to stand for fifteen minutes then filtered under suction; pH of the filtrate was altered by adding concentrated HCl; the product formed was filtered and washed with water; re-crystallized with ethanol (95%).

Yield: 63%, White colored compound, m.p: 235-237°C IR (KBr, cm^{-1}): 3440 (N-H str.), 1709(C=O str.), 3053 (C-H str., Ar-H), 1200 (C=S str.) ^{1}H NMR (DMSO) δ7.302-7.447(m 10H, 2Ar), δ11.335 (s, 1H, NH), δ 12.167 (s, 1H, NH).

Synthesis of 5,5-diphenyl-2-thioxoimidazolidin-4-one derivatives: 2a and 2b

Synthesized compound **2** (0.00125 mol) was weighed and dissolved in 20 mL ethanol in a clean 250 mL single neck round bottom flask fitted

with a reflux condenser. To this, pyridine (1 mL) and 2-chloroacetic acid (0.05 mol), **(2a)** and *p*-chlorobenzaldehyde (0.05 mol), **(2b),** were added. The mixture was refluxed for 20 h after-which it was kept standing until ambient temperature was attained; then poured into ice. The resulting precipitate was filtered using Buchner funnel with suction and re-crystallized from ethanol.

[(5-oxo-4,4-diphenyl-4,5-dihydro-1H-imidazol-2-yl)sulfanyl] acetic acid (2a): Yield: 66.2%, Pale brown colored compound, m.p: 296-298°C IR (KBr, cm^{-1}): 3440 (N-H str.), 3053 (C-H str., Ar-H), 1709(C=O str.), 1616 (C=N str.), 3000 (O-H str.) ^{1}H NMR (DMSO) δ7.330-7.418 (m 10H, 2Ar), δ9.308 (s, 1H, -OH), δ11.096 (s, 1H, NH).

4-[(5-oxo-4,4-diphenyl-4,5-dihydro-1H-imidazol-2-yl)sulfanyl]benzaldehyde (2b): Yield: 56.7%, brown colored compound, m.p: 238-240°C IR (KBr, cm^{-1}): 3440 (N-H str.), 3053 (C-H str., Ar-H), 1709(C=O str.), 1616 (C=N str.), 1745 (C=O str. Ald.) ^{1}H NMR (DMSO) δ7.303-7.446 (m 10H, 2Ar), δ7.680-7.700 (d, 2H, -Ar), δ7.925-7.946 (d, 2H, -Ar), δ10.000 (s, 1H, -C-H str. Ald.), δ11.338 (s, 1H, -NH).

Antimicrobial activity

In vitro antimicrobial study was carried on Luria agar plates (37°C, 24 h) by agar diffusion cup plate method.[23] All the compounds were screened for antimicrobial activity at 50 µg/mL concentration against the following bacterial strains: *E. coli*, *K. pneumonia* and *P. aeruginosa.* Commercial drug Ciprofloxacin was used as control. 0.001g each of the synthesized compounds were accurately weighed and dissolved in 10 mL of DMSO. 50 µL from each stock solution was dissolved in 950 µL of DMSO, to have a concentration of 50 µg/mL. Luria agar was prepared appropriately and dispensed (half filled) into 15 sterile petridishes, and allowed for about 1 h to properly gel. After gelling, the culture plates were inoculated with 50 µL of each test micro-organism and uniformly spread with a spreader and labeled accordingly. A sterile cork borer (7 mm in diameter) was used to bore wells in each plate. 50 µg/mL concentration of each solution was dispensed (half filled) into the wells. The plates were incubated at 37°C for 24 h. The zone of inhibition was measured in millimeter (mm).

Bio-safety

Antimicrobial analysis was conducted under sterile conditions. All apparatus used for preparing culture media were sterilized using an autoclave. After sterilization, the apparatus were carefully transferred into a laminar air flow and kept for about five minutes. The researcher was well kitted with laboratory apron, latex gloves and nose mask, to avoid any form of contamination. Plating, inoculation and dispensation of each sample solution were carried out in the laminar air flow to ensure safety, after which the inoculated plates were carefully transferred to the incubator.

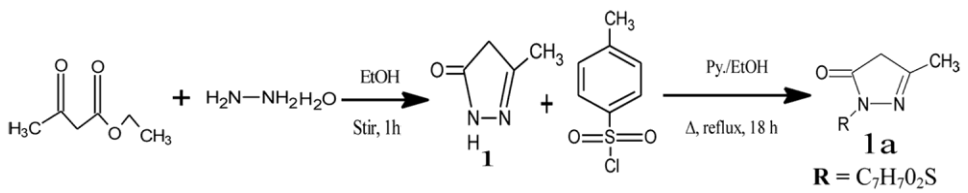
Results and Discussion

Chemical synthesis

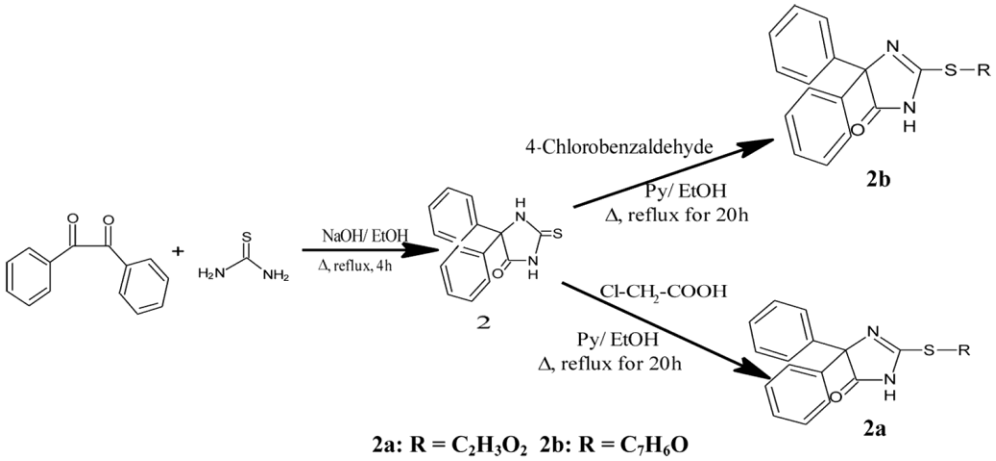
The target scaffold molecules; pyrazolone (**1**) and thiohydantoin (**2**), were synthesized by adopting well-defined synthetic methods involving cyclization reactions[21,22]. Compound **1** was prepared by the reaction between ethylacetoacetate andhydrazine hydrate in absolute alcohol. Compound **1** on condensation with *p*-toluenesulfonyl chloride in the presence of pyridine as base and ethanol as solvent, yielded compound **1a** (Scheme 1).

Compound **2** was synthesized using appropriate amounts of benzil, thiourea, 30% NaOH solution and ethanol. Separate condensation reaction between compound **2** and appropriate amount of 2-chloroacetic and *p*-chlorobenzaldehyde, in the presence of pyridine and ethanol, yielded compounds **2a** and **2b** respectively (Scheme 2).

The synthesized compounds were investigated for their biological activity. In comparison to the antimicrobial analysis of derivatives of pyrazolo [3,4-c] pyrazole[21] and thiohydantoin[14], all synthesized compounds also showed good sensitivity against the test organisms. Physical data and biological activities (zone of inhibition) of the synthesized compounds



Scheme 1 — Synthesis of 5-methyl-2,4-dihydro-3H-pyrazol-3-one (1) and 5-methyl-2-[(4-methylphenyl)sulfonyl]-2,4-dihydro-3H-pyrazol-3-one (**1a**)



Scheme 2 — Synthesis of 5,5-diphenyl-2-thioxoimidazolidin-4-one (2), [(5-oxo-4,4-diphenyl-4,5-dihydro-1H-imidazol-2-yl)sulfanyl] acetic acid (2a) and 4-[(5-oxo-4,4-diphenyl-4,5-dihydro-1H-imidazol-2 yl)sulfanyl]benzaldehyde (**2b**)

Table 1 — Physical data of synthesized compounds

Compound	R	Mol. Formula	Mol. Wt.	m.p. (°C)	Yield (%)
1	-	$C_4H_6N_2O$	98	216-218	74
1a	$-SO_2PhCH_3$	$C_{11}H_{12}N_2O_3S$	252	116-117.4	62
2	-	$C_{15}H_{12}N_2OS$	268	235-237	63
2a	$-CH_2COOH$	$C_{17}H_{14}N_2O_3S$	326	296-298	66.2
2b	-PhCHO	$C_{22}H_{16}N_2O_2S$	372	238-240	56.7

Table 2 — Zone of inhibition at 50 (µg/mL)

Compound	Zone of inhibition (mm)		
	E.coli	*K. pneumonia*	*P. aeruginosa*
1	5	9	8
1a	6	11	10
2	6	9	12
2a	8	11	10
2b	7	10	13

are summarized in Tables 1 and 2, respectively and the photos are given in Fig. S1-S5 in Supplementary Information.

Conclusion

This study entails the careful and successful synthesis, characterization and antimicrobial analysis of pyrazolone and thiohydantoin derivatives. All the compounds exhibited antimicrobial activities. The data revealed that all the tested compounds showed moderate to good inhibition in DMSO. In comparison with the synthesized derivatives, compounds **1** and **2** showed lesser sensitivity against the test organisms; compound **2b** showed the highest sensitivity against *P. aeruginosa.* Compounds **1a** and **2a** showed highest activity against *K.pneumonia* while compound 2a showed highest sensitivity against *E.coli.* It is therefore worthwhile mentioning that the integration of *p*-toluene sulfonyl chloride to pyrazolone, 2-chloroacetic acid, and *p*-chlorobenzaldehyde to thiohydandoin enhances the biological activity of the lead compounds. These preliminary results of biological activities offer an encouraging framework that may lead to the discovery of novel antimicrobial agents.

Supplementary Information

Supplementary information is available in the website http://nopr.niscpr.res.in/handle/123456789/58776.

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