Murrayanine-1,3,4-Oxadiazole-Uracil Hybrid: The Emerging Anti-inflammatory Candidate

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Abstract - Inspiring from the hybridization approach, a hybrid 6-(((5-(1-methoxy-9*H*-carbazol-3-yl)-1,3,4-oxadiazol-2-yl)amino)methyl)pyrimidine-2,4(1*H*,3*H*)-dione (3) was rationally designed and fabricated by incorporating murrayanine, 1,3,4-oxadiazole, and uracil components and screened for the exploration of the amplified anti-inflammatory potential by utilizing the carrageenan-induced paw edema method. The fabricated novel hybrid displayed significant edema reducing potential in the carrageenan-induced paw edema model as evidenced by 24.33%, 40.57%, and 59.99% reduction in the inflammation in the three individual hrs. On comparing the anti-inflammatory data of the previously developed murrayanine-oxadiazole derivative, it was observed that a significant decrease in the edema in rats has occurred with the addition of the uracil moiety to the present scaffold. It might be predicted from the present research that the reduction in edema in the rats may be due to the inhibition of inflammatory mediators like cyclooxygenase-1/2 (COX-1/2) and lipoxygenase (LOX) (primarily) by interacting with the active sites through the carbonyl, methoxy, and amide groups. The study will open new avenues of research for the modern age researchers in the rational designing of hybrid molecules with significant anti-inflammatory activity.

Keywords: Murrayanine; *Murraya koenigii*; Oxadiazole; Uracil; Anti-inflammatory; Hybrid.

Introduction

As the time is rapidly progressing, the upcoming challenges to the present human health and mankind have necessitated the researchers for developing better alternatives.[1] The area of rational drug design and development is mushrooming with a focus for developing the best method and molecule with highest pharmacotherapeutics.[2] The amplified benefits are the result of several recently developed approaches which are based on the basic structures of the subject.[3] Hybridization is one of the most promising approaches for the rational fabrication of the novel molecules by incorporating various pharmacologically active scaffolds (with prominent active groups for interaction) where the cumulative effect often produces a molecule with tremendous therapeutic potential.[4]

Murrayanine is a pharmacologically active carbazole present in the root, stem, and leaves of Indian curry plant, known as *Murraya koenigii* L. (Family: Rutaceae).[5] The phytoconstituent have therapeutic values like anti-oxidant, anti-bacterial, anti-fungal, antiseptic, etc., which are quite low and is not a very emerging molecule in modern day medicine. After knowing the fact, we had rationally fabricated numerous semi-synthesized hybrids of murrayanine (chalcone, thiadiazole, oxadiazole, benzodiazepine, pyrimidine, isoxazole, benzoxazepine, hydantoin, pyrazole, benzothiazepine, phthalimide, thiazole, methylsulfone, Schiff's base derivative, imidazole, hydroxylated chalcone, and 3,4-methylenedioxy) with a desire that the novel produced compounds will have better, amplified, and important therapeutic value.[6-22]

Inspiring from the above approach, a hybrid was rationally designed and fabricated by incorporating murrayanine, 1,3,4-oxadiazole, and uracil components and screened for the exploration of the amplified anti-inflammatory potential by utilizing the carrageenan-induced paw edema method.

Materials and Methods

Chemical and Instrumentation

6-(chloromethyl)pyrimidine-2,4(1*H*,3*H*)-dione **(2)**, the (reactant) was purchased from Sigma-Aldrich®, Germany through a local vendor in Nagpur. 5-(1-methoxy-9*H*-carbazol-3-yl)-1,3,4-oxadiazol-2-amine **(1)** was obtained from our previous report.[16] The progress of the chemical reaction was monitored by using the Merck® silica gel G-coated thin layer chromatography plates. The destination compound was confirmed by the

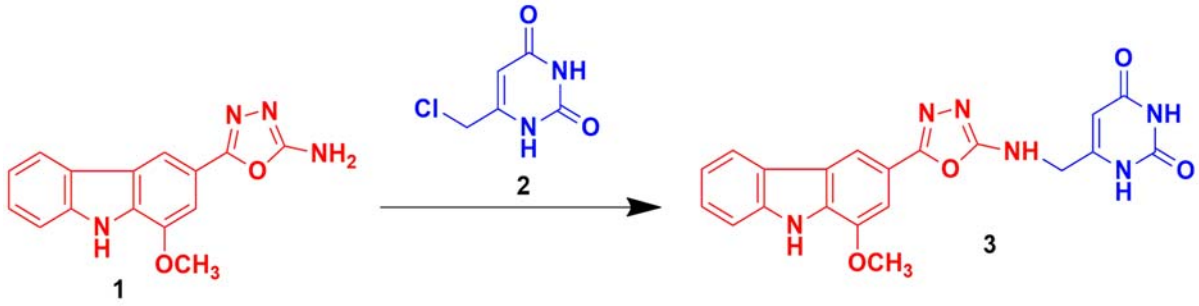
sophisticated spectroscopic technique like KBr discs based Fourier-transformed infrared (FT-IR) (IRAffinity-1), mass technique (JEOL-JMS-DX 303), and ^{1}H-NMR technique (Bruker spectrospin NMR DPX-300) using internal standard tetramethylsilane. Elemental Analyzer (Perkin-Elmer 240C) was employed for the elemental analysis.

Animals

After procuring the procuring permission from CPCSEA (1389/a/10/CPCSEA) and Department Ethical Committee (DEC), the *in vivo* anti-inflammatory study of the hybrid **(3)** was evaluated in Swiss male albino rats of 5-6 week age and 135-225g weight. The experimental animals were placed in the animal house during the study under following controlled environment (free access to water, provided with standard rodent pellets, maintained under 24–25ºC temperature and 50–60% humidity, and following the 12 hrs cycle of light and dark).

Synthesis of target compounds

The synthesis of the final compound, 6-(((5-(1-methoxy-9*H*-carbazol-3-yl)-1,3,4-oxadiazol-2-yl)amino)methyl)pyrimidine-2,4(1*H*,3*H*)-dione **(3)** involved reaction of 5-(1-methoxy-9*H*-carbazol-3-yl)-1,3,4-oxadiazol-2-amine **(1)** and 6-(chloromethyl)pyrimidine-2,4(1*H*,3*H*)-dione **(2)** in the presence of triethylamine at the room temperature. Here, the reaction involved the transformation of amine portion into amide, in which, abstraction of proton took place from the starting material **(1)**. The corresponding chloride moiety from the reactant **(2)** gets eliminated to form hydrochloride (HCl). The triethylamine is a good nucleophile and maintains the neutrality of the reaction media. The chemical reaction is depicted in **Scheme 1**.



Scheme 1. The synthetic approach to murrayanine-oxadiazole-uracil hybrid.

Synthetic protocol for 6-(((5-(1-methoxy-9H-carbazol-3-yl)-1,3,4-oxadiazol-2-yl)amino)methyl)pyrimidine-2,4(1H,3H)-dione **(3)**

The experiment was performed in a three-neck flask where 0.01 M of ethanol-methanol solution of 5-(1-methoxy-9*H*-carbazol-3-yl)-1,3,4-oxadiazol-2-amine **(1)** was taken and triethylamine was dropwise added. The above content was stirred at high RPM for the duration of 20 min. Later, methanolic solution of 0.01 M of uracil **(2)** was added and the produced reaction content was stirred at low RPM on a magnetic stirrer for 30 min. The produced hybrid compound was obtained by pouring the reaction content in a thin stream over the crushed ice to separate out the final hybrid compound **(3)**, which was subsequently washed, dried, and recrystallized.

66% yield; FTIR (KBr) υ (cm^{-1}): 3163 (-NH, stretching), 3071 (C-H, aromatic), 1735 (C=O, stretching), 1692 (C=N, five-membered), 1669 (C=C, aromatic), 1602 (-NH, bending), 1491 (-CH_2, bending), 1282 (C-N, stretching), 1233 (C-O, stretching); ^{1}H NMR (δ, ppm, $CDCl_3$): 10.12 (9, Carbazole, 1H), 10.03 (18, Uracil Amide, 1H), 7.2-8.3 (Aromatic, 6H), 6.23 (16, Uracil Amide, 1H), 3.87 (14, Methylene, 2H), 4.15 (13, Amide, 1H), 3.99 (1, 3H). MS: M^+ 404. Anal. Calcd. for $C_{20}H_{16}N_6O_4$: C, 59.40; H, 3.99; N, 20.78. Found: C, 58.76; H, 3.14; N, 20.32

Acute toxicity studies

The *in vivo* acute toxicity of the hybrid was estimated according to the OECD guideline to explore the maximum therapeutic effect with no considerable toxicity. The protocol involved administrating the compound at escalating dose ranging from 25 mg/kg to 500 mg/kg. The therapeutic dose was computed based on LD_{50} values.[23]

Anti-inflammatory screening

The *in vivo* anti-inflammatory activity of the murrayanine-oxadiazole-uracil hybrid was evaluated using the carrageenan-induced paw edema method. Before starting the protocol, the Swiss albino rats were fasted overnight and individually fed with distilled water (5 mL) to all the subjects. The hybrid molecule was screened at a dose of 100 mg/kg b.w. before the commencement of the inflammation. The inflammation was produced in the right hind paw of the rat at the subplanter region by injecting 1% carrageenan solution. The control group was given saline solution containing solubilizer. By utilizing the mercury digital micrometer, the thickness of each paw of the rats was measured for the duration of 3 hrs with an interval of 1 hr. The disparity between the

width of injected and non-injected paws was estimated for the exploration of anti-inflammatory ability of the hybrid. Indomethacin (10 mg/kg b.w.) was utilized as the positive control. The obtained data were expressed as the Mean ± SEM. [24]

Statistical treatment

The obtained anti-inflammatory data were analyzed statistically by one-way ANOVA approach followed by Dunnett's multiple comparison test. The P-value < 0.01 was considered to be statistically significant.

Result and Discussion

Chemistry

The spectroscopic studies have revealed the proposed structure of the murrayanine-1,3,4-oxadiazole-uracil hybrid **(3)**. The formation of amide or in another way, the attachment of uracil portion **(2)** with the murrayanine-oxadiazole **(1)** was confirmed from the FT-IR spectrum where the amine component ($–NH_2$ peak) which was present in the earlier FT-IR spectrum at 3375 cm^{-1}, got vanished in the spectra of the hybrid. Additionally, the C-N portion between the oxadiazole linked amide and the methylene carbon was seen at 1282 cm^{-1}. Furthermore, the fabrication of the hybrid was ascertained from the amide proton (at position 13) at 4.15 ppm in the NMR spectrum. Moreover, the formation of $–CH_2-$ linkage was located at 1491 cm^{-1} in FT-IR spectrum and at 3.87 ppm in ^{1}H-NMR. The attachment of the uracil scaffold with the murrayanine-1,3,4-oxadiazole scaffold was discovered by the –NH moieties in the positions 16 and 18 in the NMR spectrum at 6.23 ppm and 10.03 ppm. The presence of 1,3,4-oxadiazole was validated from the C=N moiety at 1692 cm^{-1}. The carbazole –NH was detected at 10.12 ppm. The aromatic rings with the carbazole moiety were predominantly observed in the range of 7.2-8.3 ppm. The other features of the ring like C-H and C=C were chiefly substantiated at 3071 cm^{-1} and 1669 cm^{-1} in FT-IR spectrum. Besides, the elemental analysis revealed that the practically obtained values were in close agreement with the theoretical % composition of the elements, which supported the formation of the novel fabricated compound. The mass spectra displayed that the base peak corresponds precisely with the theoretical molecular mass (404), also with the appearance of few fragment peaks.

Anti-inflammatory screening

The fabricated novel hybrid **(3)** displayed significant edema reducing potential in the carrageenan-induced paw edema model as evidenced by 24.33%, 40.57%, and 59.99% reduction in the inflammation in the three individual hrs (**Table 1**). On comparing the anti-inflammatory data of the previously developed murrayanine-oxadiazole derivative, it was observed that a significant decrease in the edema in rats has occurred with the addition of the uracil moiety to the present scaffold. It might be predicted from the present research that the reduction in edema in the rats may be due to the inhibition of inflammatory mediators like cyclooxygenase-1/2 (COX-1/2) and lipoxygenase (LOX) (primarily) by interacting with the active sites through the carbonyl, methoxy, and amide groups.

Table 1. Result of the *in vivo* anti-inflammatory screening of murrayanine-oxadiazole-uracil hybrid.

Compound	Percentage (%) inhibition of edema		
	1 hr	2 hr	3 hr
3	24.33* ± 2.53	40.57* ± 2.24	59.99** ± 2.37
Indomethacin	35.51* ± 1.74	57.61* ± 1.43	78.32** ± 1.89

n = 6; ED_{50} of 100 mg/kg b.w. in male adult albino mice; **P < 0.01; *P< 0.05

Conclusion

The present study revealed that hybridization is an amazing way for developing better pharmacological analog. The incorporation of the uracil component in the previously developed murrayanine-oxadiazole derivative has resulted in the amplification of the *in vivo* inflammatory activity in carrageenan-induced paw edema model (59.99% in 3 hrs). It might be predicted from the present research that the reduction in edema in the rats may be due to the inhibition of inflammatory mediators like cyclooxygenase-1/2 (COX-1/2) and lipoxygenase (LOX) (primarily) by interacting with the active sites through the carbonyl, methoxy, and amide groups. The study will open new avenues of research for the modern age researchers in the rational designing of hybrid molecules with significant anti-inflammatory activity.

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